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HANDBOOK OF
CHEMICAL MICROSCOPY

IN PREPARATION

VOLUME II

Chemical Methods and Inorganic Qualitative Analysis

HANDBOOK OF CHEMICAL MICROSCOPY

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Volume I

Principles and Use of Microscopes and Accessories
Physical Methods for the Study of Chemical Problems

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PREFACE

For many years there have been given in the Department of Chemistry of Cornell University a number of courses designed to acquaint our students with microscopes, their accessories, and their applications to the solution of laboratory and works problems. These courses, both elementary and advanced, have consisted of lectures and laboratory practices, emphasis being laid upon the basic principles involved in intelligently applied microscopy, rather than upon the study of special classes of materials.[1]

In the early days, attention was concentrated upon the practical applications of microscopic methods and especially their adaptability to the analysis of very minute amounts of material; this was the keynote of *Elementary Chemical Microscopy*, written in 1914 to provide students with a textbook in the English language.

As time passed it became evident that a somewhat broader treatment was desirable, covering methods and principles of optical and other physical tests applicable to the examination of industrial materials and to the investigation of physico-chemical phenomena. In many fields of industry, new types of problems created a demand for new methods of attack, and gave rise to important advances in microscopical technique and extensions of its applications. The usefulness of the microscope for the demonstration of many of the abstract principles of physical chemistry encouraged us to expand this phase of the instruction as a means of supplementing other courses in chemistry and of elucidating material ordinarily presented only by graphs or tabulations of data.

These developments in the field of chemical microscopy, together with the theoretical background necessary for an understanding of them, have hitherto been discussed mainly in advanced lecture and demonstration courses. Students desiring to employ special procedures not covered in the elementary course, industrials who availed themselves of our laboratory facilities to pursue an investigation, or workers in other fields of science who wished to take advantage of the methods of chemical microscopy have not had available a course of reading and laboratory experimentation which would quickly acquaint them with the fundamentals essential to the proper prosecution of their researches.

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[1] The educational features of chemical microscopy and its value in the training of chemists are discussed by Chamot and Mason: *Jour. Chem. Education* **5**, 9, 258, 536 (1928).

The present *Handbook of Chemical Microscopy* is an attempt to present in logical and concise form the principles and methods involved in practical microscopy. To keep the book within suitable bounds, a rather comprehensive bibliography is incorporated, in order that the investigator may better prepare himself to attack any one of the countless problems which confront him.

Although based on *Elementary Chemical Microscopy*, the present work has been entirely rewritten, and expanded in all sections. The optical principles of the microscope have been presented in some detail, as a preliminary to the discussions of the special technique of illumination, photomicrography, and ultramicroscopy. The factors underlying the interpretation of appearances in microscopic objects are formulated in general terms, as is the discussion of the properties of doubly refractive material, in order that they may lend themselves to application in various fields of microscopy. In line with the rapidly widening applications of crystallographic principles, their fundamental concepts are exemplified by numerous experiments which have been devised primarily from the point of view of the chemist.

Much of the material here compiled and discussed for the first time in book form is of paramount importance to all microscopists whether they are workers in the biological or in the physical sciences. This book may therefore as well be called a Handbook of Applied Microscopy.

Volume I has been confined to a discussion of optical principles of instruments, manipulative methods of general application, and the observation of physical and physico-chemical phenomena. Copious references have been given to journal articles or books wherein can be found detailed directions for specific microscopical methods.

Volume II is devoted to chemical reactions as studied under the microscope, with particular reference to inorganic qualitative analyses, emphasis being laid upon the time- and labor-saving features of the methods rather than upon the supersensitivity of the selected identity tests.

The authors wish to acknowledge their indebtedness to the many investigators who have published researches involving microscopical methods; we have drawn freely from this material, but have endeavored to give due credit in every case.

To the Bausch & Lomb Optical Co., E. Leitz, Inc., and the Spencer Lens Co., the authors are indebted for the loan of instruments and apparatus and also for their ever friendly coöperation in the development of new instruments or the modification of old types better to meet the needs of chemical microscopy.

To Dr. L. M. Dennis, Director of the Department of Chemistry of Cornell University, the authors are especially deeply indebted, for without his sympathetic interest and generous support during the past thirty years, it would have been impracticable to develop and extend courses of instruction in chemical microscopy and impossible to have obtained the extraordinarily complete equipment now housed in the Baker Laboratory of Chemistry.

E. M. C.
C. W. M.

Ithaca, N. Y., *June*, 1929.

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HANDBOOK OF CHEMICAL MICROSCOPY

INTRODUCTION

The term **chemical microscopy** was coined by the senior author many years ago, for the purpose of differentiating between " microchemical " methods and tests and the application of the microscope to the solution of chemical problems. Microchemistry implies chemistry on a small scale; formerly this term referred to qualitative analytical reactions as observed under the microscope or to modifications of ordinary quantitative analytical procedures such as those devised by Pregl and others to deal with very minute amounts of material. At present, however, there is a growing tendency to designate as " microchemical " all highly sensitive identification reactions and all quantitative methods which permit the use of samples smaller than are commonly necessary in " standard methods." This extension of the field of " microchemistry " to include spectroscopic and colorimetric analyses, biological reactions, and other wholly unrelated analytical methods is sufficient vindication for the term chemical microscopy and justifies assembling under this head those methods, principles, and phenomena of chemistry which may be studied particularly advantageously by means of the microscope — not because they exemplify the manifold uses of the instrument, but because the technique, theoretical foundation, and interpretation of microscopical studies are all closely interrelated, whatever the materials examined.

Chemical microscopy claims recognition more because it yields observations which are direct and vivid, conclusions which are more positive, and results which are often unobtainable by other methods, than because only minute amounts of material are necessary for study. Although its methods do not always involve chemical reactions, they do yield chemical information, and they require chemical common sense in their application and interpretation.

The enormous and ever-growing diversity of problems susceptible of solution by microscopical methods precludes the development of fixed rules of procedure which may be followed empirically. The microscopist must be continually guided by the basic principles which underlie the field and which, though few in number, are of the widest usefulness in all phases of microscopy. He must be alert to utilize these fundamental concepts in widely different connections, and to unify his experiences in terms of their theoretical foundation.

First and foremost, the worker must know his tools. The microscope is not an automatic or self-sufficient instrument, but an accessory to the eye and brain of the investigator. As an instrument, it must be manipulated with skill and facility; as an aid to observation, its limitations and the optimum procedures for its use must be continually borne in mind; as an apparatus for the determination of optical properties as well as for mere magnification, the origin and significance of the phenomena observed must be understood. The discussion of the theoretical principles of microscopy given in the following pages has been restricted to a minimum, and presupposes only an elementary knowledge of optics. However, a thorough comprehension of this minimum is essential, and is indispensable as a foundation for general work.

The simple laboratory practices which are given, and other experiments which will suggest themselves to the student, are necessary preliminary exercises in manipulation and in the interpretation of appearances. Without this experience, the worker is liable to serious mistakes, especially when attempting to translate the novel and changing phenomena of an optical image in terms of their chemical significance. Simple tests and observational technique, intelligently employed and conservatively interpreted, are far more trustworthy than ill-considered, elaborate, empirical procedures, which may conceal pitfalls and lead to hasty and usually faulty conclusions. Frequently more than one interpretation is possible, and the investigator should not lose sight of the purely objective phenomena observed, or obscure the actual experimental facts in the midst of extended descriptions which are largely matters of opinion. The conditions under which these phenomena are exhibited also constitute an essential part of the record, and cannot be reported too explicitly.

In addition to the practices suggested, the worker who desires to train himself in the methods and applications of chemical microscopy should follow as far as possible the reading suggested in connection with the phenomena and procedures under discussion. As the knowledge thus acquired engenders skill and confidence in the instruments and technique employed he will be able to study more intelligently the work of others in the field which interests him. Eventually his reading should extend to include the chief work in fields of microscopy apparently unrelated to his own, which he will be able to evaluate in terms of their adaptability to his investigations. The reader of the following chapters will find that the methods and principles of chemical microscopy are not necessarily peculiar to chemistry but, as their origins indicate, are common to all fields of microscopy. The microscopist working in the biological sciences may therefore derive profit from the experiments and accompanying reading equally with the chemist.

The references cited have been selected for their value as introductions to the general fields of microscopy which they cover, and many will be found to contain extensive bibliographies for further study. Where specific technical applications are mentioned, only the most fundamental works and the most complete discussions of general methods are given. As a consequence, the literature on the microscopy of individual technical products is far from complete, and the references are given more for the benefit of workers in other fields than for specialists. It is hoped that they will be suggestive of technique or applications, and that they will be regarded as the basis of modifications to suit the particular problem at hand, rather than as rigid directions to be followed.

In certain long-established special fields of applied microscopy the methods, interpretations, and the literature are so well defined that they are readily accessible in standard works. For this reason the uses of the microscope in the biological sciences, mineralogy, and metallography are hardly touched upon in this present work, and no attempt has been made to give complete bibliographies in these fields. Other technical applications are discussed in the books listed in the bibliography on page 447.

CHAPTER I

THE OPTICAL SYSTEM OF THE MICROSCOPE

In chemistry and technology the microscope is utilized for two kinds of studies: the revelation of fine structure, and the determination of optical and other properties of substances. In either case, the observations are primarily based upon *optical* phenomena, and are limited or extended by the optical system of the microscope.

Ingenious design and sound construction of the mechanical features of the stand of the instrument may add greatly to the convenience and precision of manipulation; on the other hand, a clumsy and poorly made stand may be a great hindrance to many essential microscopic operations. Ultimately, however, the quality of the lens system is the real measure of the usefulness of the microscope, for it determines the perfection of the microscopic image which is obtained, and the accuracy with which this enlarged image represents the actual structure of the minute object under examination.[1]

---

[1] The optical system of the microscope and other general phases of microscopy are discussed in the following books:

Beck, C.: *The Microscope.* Parts I and II. (R. & J. Beck, London, 1923–24.)

Carpenter, W. B.: *The Microscope and its Revelations* (Churchill, London, 1901).

Gage, S. H.: *The Microscope* (Comstock Publishing Co., Ithaca, N. Y., 1925).

Köhler, A.: *Das Mikroskop.* Abderhalden's *Handbuch der biologischen Arbeitsmethoden,* Abt. II, Hft. 2, pp. 171–352 (Urban & Schwarzenberg, Berlin, 1923).

Krause, R.: *Enzyclopädie der mikroskopischen Technik.* Bd. II (Urban & Schwarzenberg, Berlin, 1926).

Langeron, M.: *Précis de Microscopie* (Masson, Paris, 1925).

Marshall, C. R., and Griffith, H. D.: *Introduction to the Theory and Use of the Microscope* (Routledge, London, 1928).

Metzner, P.: *Das Mikroskop* (F. Deuticke, Leipzig, 1928).

Spitta, E. J.: *Microscopy* (E. P. Dutton, New York, 1920).

Wright, A. E.: *Principles of Microscopy* (Constable, London, 1906).

Disney, A. N., ed.: *The Origin and Development of the Microscope* (Royal Microscopical Society, London, 1928).

The **lens system** of a compound microscope consists of an objective and an eyepiece, both of which function in the production of the image. A third lens, the condenser, may be used to illuminate the object (Fig. 1).

IMAGE FORMATION AND MAGNIFICATION

From Fig. 1 it will be seen that the object F_1 is placed just outside the lower focal plane of the objective. This setting is necessary in order that the objective shall form a *real* image of the object. The position of this real image is determined by the focal length of the objective and the distance at which the object is placed. The objective may be raised or lowered until it is at the proper distance from the object to form a real image F_2 at the focal plane of the eyepiece; this operation is called **focusing the microscope.**

The size of the real, inverted image is determined solely by the *relative distances* of object and image from the objective. This relationship may be expressed by either of the following formulas for the magnification by the objective:

$$(1)\quad \frac{\text{length of image}}{\text{length of object}} = \frac{\text{distance of image from back focal plane of objective}}{\text{focal length of objective}}$$

$$= \frac{\text{“optical tube length”}}{f\ \text{objective}}$$

$$(2)\quad \frac{\text{length of image}}{\text{length of object}} = \frac{\text{distance of image from “effective center”}^{2}\text{ of objective}}{\text{distance of object from “effective center” of objective}}$$

For an objective of any given focal length, the size of the image (and therefore the magnification) can be varied by altering the distance at which this image is formed, in accordance with the above relationships. By moving the eyepiece farther away from the objective (increasing the " tube length ") and refocusing so that the image is again formed within the eyepiece, the size of the image is increased. This adjustment permits varying the magnification of the microscope *gradually* over a range of about 25 per cent by increasing or decreasing the mechanical tube length between the usual limits of 140 to 180 mm.

Manufacturers have recently begun to mark objectives not only with their focal length but also with their " magnification

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[2] See p. 22.

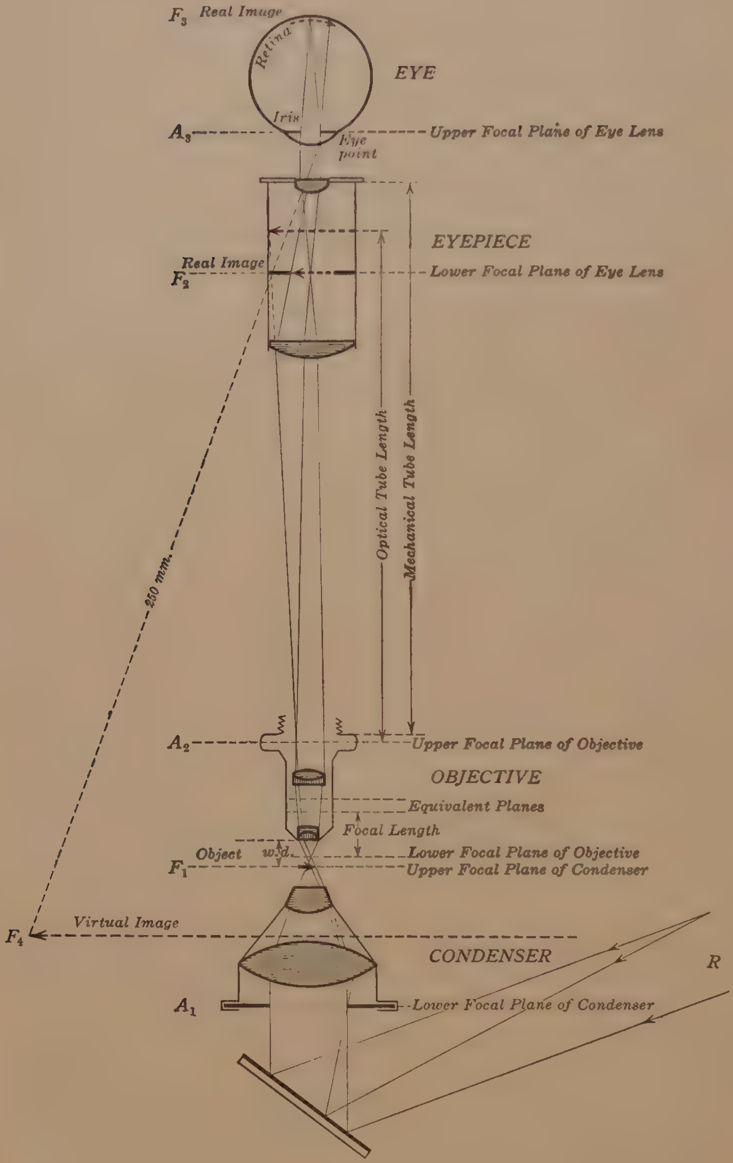
F3
Real Image
Retina
EYE
Iris
A3
Upper Focal Plane of Eye Lens
Eye point
EYEPIECE
Real Image
F2
Lower Focal Plane of Eye Lens
Optical Tube Length
Mechanical Tube Length
250 mm.
A2
Upper Focal Plane of Objective
OBJECTIVE
Equivalent Planes
Focal Length
Object
w.d.
Lower Focal Plane of Objective
F1
Upper Focal Plane of Condenser
Virtual Image
F4
CONDENSER
R
A1
Lower Focal Plane of Condenser

FIG. 1. The Optical System of the Microscope.

numbers." These are calculated by means of the formulas given, usually on the basis of a mechanical tube length of 160 mm.

The real image formed by the objective is focused at (or very slightly above) the focal plane of the upper lens of the eyepiece. Acting jointly with the lens of the eye, the eyepiece forms a so-called **virtual image** F_4 of the real image from the objective. The image from the objective is therefore not re-inverted by the eyepiece. This virtual image does not exist in space, but is actually a real image on the retina F_3, and it may appear near or distant depending on slight adjustments of the focus of the microscope and of the eye.

Because of its subjective character, the size of such a virtual image can best be expressed in terms of its apparent size at the "normal distance of close vision," which is arbitrarily taken as 10 inches or 250 mm. The eyepiece, therefore, effects a magnification which may be expressed by a formula similar to (2)

$$(3)\quad \frac{\text{length of virtual image}}{\text{length of ``object''}} = \frac{\text{``distance of close vision''}}{\text{focal length of eyepiece}} = \frac{250}{f\ \text{eyepiece}}$$

Eyepieces are commonly marked with a "magnification number," which is determined from this formula.

It is evident that the image formation and magnification of the compound microscope depend on the combined action of objective and eyepiece. The magnification of the instrument as a whole is given by the following formula:

$$(4)\quad \text{magnification} = \frac{\text{tube length}}{f\ \text{objective}} \times \frac{250}{f\ \text{eyepiece}}$$

This is equivalent to the product of the magnification numbers of objective and eyepiece, for the particular tube length specified by the maker.

Such a formula is mathematically accurate, but it is only approximate in practice because the focal lengths marked on lenses are usually given only to one or two significant figures, and the mechanical tube length, as read from the draw-tube graduations, does not correspond to the actual optical tube length for all objectives and eyepieces. The formula is of great value in rapid approximations of the magnifications obtainable with any given lenses, and because it summarizes the variables which determine the magnification in any case. It is accurate enough for visual work which does not involve micrometry; for microscopic measurements and photomicrography more exact methods of determination of magnification are available (pages 255, 395).

The rangé of magnifications used in microscopy is often divided roughly as follows: low power, 10 to 100 diameters; medium power, 100 to 500 diameters; high power, 500 to 1500 diameters or even higher.

OBJECTIVES

Of all the lenses of the microscope, the objective is of greatest importance, for its properties make or mar the final image, and influence markedly the convenience of manipulation.

The main functions of an objective are:

1–To gather the light coming from any point of the object.
2–To unite this light in a point of the image.
3–To form the image at such a distance that magnification is obtained.

Light-Gathering Properties of Objectives. — Any illuminated object, whether self-luminous or not, sends out light in all directions. For examination of its coarser features, a small portion of this radiation is sufficient, but for the revelation of finer details it is necessary that the light utilized shall come from the object in as wide a range of directions as possible. This is accomplished by the use of lenses which include a cone of light rays and transmit them to form the image. Objectives of long focal length would have to be of inconveniently large diameter to accomplish this, but objectives of short focal length can be constructed so as to include a very wide-angled cone of rays. It has long been known experimentally that the angular magnitude of the cone of rays "grasped" by the objective gives an indication of its power to reveal fine structure in the object.

The angle between the most divergent rays which can pass through the objective to form the image is called the **angular aperture** (**A.A.**), and this value was long used as a means of quantitative comparison of objectives. The maximum angular aperture possible is of course 180°.

Numerical Aperture. — With the development of "immersion" objectives (page 23), which are used with liquid between their front surface and the object, it became evident that angular aperture was not the sole factor which governed the ability of an objective to resolve structure into its finest details. An immersion objective, of only moderately high angular aperture, actually could reveal finer detail than " dry " objectives of much greater angular aperture. This discrepancy between resolving power and notation in terms of angular aperture was finally cleared up by Ernst Abbe, who showed that the index of refraction (n) of the immersion medium must be taken into account. He proposed the term **numerical aperture** (**N.A.**).

$$\text{N.A.} = n \times \sin\frac{\text{A.A.}}{2}$$

This formula is based on the fact that light entering a medium will follow identical paths, whatever the angle of incidence, if the product of the sine of this angle and the refractive index of the medium from which light enters is constant.

Numerical aperture has been shown by experiment and by various methods of calculation to be a true measure of resolving power, and is now universally employed as a means of rating objectives.[3]

Diffraction Theory of Resolution. — The **resolving power** of a microscope objective may be defined as its ability to reveal closely adjacent structural details as actually separate and distinct. Quantitatively, it may be expressed as the minimum distance between such details, when resolution is achieved. On the basis of a study of diffraction gratings and other periodic fine structures, Abbe advanced a theory of resolution which helps to correlate the various factors which govern the resolving power of microscopic objectives.

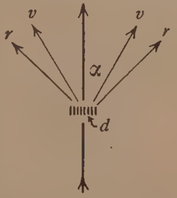

FIG. 2. Diffraction of Light by a Fine Periodic Structure.

It is known that if a diffraction grating is illuminated by a beam of light perpendicular to its surface, spectra are formed on either side of the path of the directly transmitted ray. The angle α which the diffracted rays make with the central beam depends on the distance d between the lines of the grating in comparison with the wavelength of light λ, in a medium of refractive index n (Fig. 2).

---

[3] Methods of determining the numerical apertures of objectives are given by Wright: *Principles of Microscopy* (1906), p. 197; Metzner: *Das Mikroskop* (1928), pp. 306–311; Hartridge: *Jour. Roy. Micros. Soc.* **1918**, 337.

The formula for the direction of the first diffracted ray is

$$n \cdot \sin \alpha = \frac{\lambda}{d}$$

A closely ruled grating sends off diffraction spectra at wider angles than does a coarse one. A grating diffracts red light at a wider angle than it does blue, because its spacing is finer in comparison with red light than it is in comparison with the shorter wavelengths. A grating surrounded by a highly refractive medium diffracts light through a smaller angle than it does in medium of low refractive index, because the wavelength of light is shortened in a medium of high refractive index. These facts about diffraction may be observed with the naked eye, or by means of a lens, as shown in Fig. 3.

A replica grating of 14,000–25,000 lines per inch, illuminated by an intense and narrow beam of light perpendicular to its surface, will give off well defined diffracted rays on either side of the direct beam. Their paths may be revealed by holding over the grating a piece of ground glass, or placing on it a cube of uranium glass.[4]

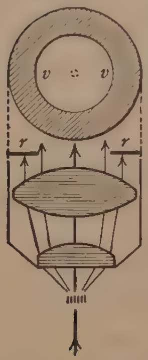

FIG. 3. Diffracted Rays at the Back Aperture of an Objective.

Abbe found that for the most perfect resolution of the structure of a grating it was necessary that all the diffracted rays should be included within the angular cone of the objective, and reunited in the image. He also found that the structure would not be resolved at all unless at least two rays (wavefronts) were included by the objective and converged by it to unite at the image.[5] A large angular aperture is therefore essential for high resolving power. The maximum angular aperture possible is 180°, and if a grating is so fine that its diffracted rays make an angle of 90° on either side of the directly transmitted beam, it will be at the limit of resolution of the microscope *for illumination parallel to the axis of the instrument.* This limit is equivalent to the wavelength of the light used, or, for any angular aperture less than 180°, to $\dfrac{\lambda}{\sin \dfrac{A.A.}{2}}$.

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[4] Schmelik: *Zeits. wiss. Mikros.* **37**, 97 (1920).

[5] Discussion and demonstrations of diffraction phenomena in microscopic vision are given by Porter: *Phil. Mag.* (6) **11**, 154 (1906). See also Pulfrich: *Zeits. wiss. Mikros.* **38**, 264 (1921).

A Leitz 5.4-mm., 0.74-N.A. objective with iris diaphragm may be used to duplicate some of Abbe's experiments. If this lens is focused on a replica grating of 25,000 lines per inch, illuminated by a very narrow axial beam of light, the lines will be resolved. If the eyepiece is removed the diffracted rays may be observed as spectra on either side of the back aperture of the objective (Fig. 3). If the diaphragm is closed just sufficiently to cut off these diffracted rays the resolution vanishes.

With a coarser grating (14,000 lines per inch) more diffracted rays are included by the objective, but decreasing its aperture until these are excluded destroys the resolution.

Increased Resolution with Light of Short Wavelength. — By using light of short wavelength the resolving power of the microscope may be increased considerably. This may be done by means of blue or blue green color screens, for visual microscopy. In photographic work, ordinary plates accomplish this effect because their maximum sensitivity lies in the violet or ultraviolet region of the spectrum. Ultraviolet light, of wavelength 275 mμ, is used to give nearly double the resolution possible with white light.[6] Quartz objectives are necessary, and they are constructed for use with strictly monochromatic light. (See page 103.) The actual resolving power of a "monochromat" of 1.25 N.A. corresponds to an effective numerical aperture of nearly 2.5.

The objective and grating used above will serve to demonstrate the influence of color on resolving power. The iris diaphragm may be closed so as to cut off all but the inner (violet) portion of the two diffraction spectra. Under these circumstances the rulings will be resolved if blue light is used, but will be invisible with red light.

Increased Resolution with Immersion Objectives. — The importance of refractive index in the expression for numerical aperture is evident from the diffraction theory, since the wavelength of light is shorter in a highly refractive medium. This is the basis of the superior resolving power of **immersion objectives** (page 23). By surrounding the object with a material of high refractive index, the diffracted rays are included within a smaller angle than if the object were in air. If the entire path from object to objective is through highly refractive media, an objective of a given angular aperture will grasp more diffracted rays (or the first diffracted ray from a finer structure) than if it were used with an air space between it and the preparation.[7]

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[6] Köhler: *Z. wiss. Mikros.* **21**, 129–65, 273–304 (1904).

Diffracted rays which are barely grasped by a " dry " objective of maximum numerical aperture (0.95), and which occupy a cone of over 140° in air ($n = 1.0$), will occupy only a cone of about 90° in a medium of $n = 1.5$. Structures well beyond the limit of resolution of such a dry objective may be easily resolved by an immersion objective of maximum numerical aperture (1.40), which corresponds to an angular aperture of 134° in a medium of refractive index 1.5.[8]

Various media have been used in conjunction with specially designed objectives, to increase resolution according to this principle. Although the theoretical maximum N.A. is $n \times \sin 90°$, ($n \times 1.0$) angular apertures of 180° are not feasible to construct, and the N.A. of the best objectives is always somewhat less than the n of the immersion medium. The usual limits are: water immersion, N.A. = 1.25, $n = 1.33$; glycerine immersion, N.A. = 1.25, $n = 1.46$; cedar wood oil (homogeneous) immersion, N.A. = 1.40, $n = 1.515$; α-monobromnaphthalene immersion N.A. = 1.60, $n = 1.658$.

Increased Resolution with Oblique Illumination. — According to Abbe's theory, the direct ray and *one* of the diffracted rays should be sufficient to resolve a grating. By the use of an oblique illuminating beam, these two rays may both be grasped by the objective when otherwise only the direct ray would enter (Fig. 4).

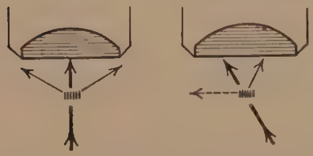

FIG. 4. Effect of Oblique Illumination on the Inclusion of Diffracted Rays by an Objective.

With axial illumination, a grating of 25,000 lines per inch is barely resolved by an 8-mm., 0.50-N.A. objective (or one of 14,000 lines per inch by a 16-mm., 0.25-N.A. objective).

Rendering the illumination oblique, so as to fall across the lines of the gratings, the resolution is much improved. The diffraction spectra are only partially included by the objective, with axial light. With oblique light, they may be seen to have shifted laterally, so that the direct beam and one spectrum are completely included by the objective.

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[7] Such a comparison is not directly possible in practise, however, for any given objective must be specially constructed for use either "dry" or "immersed," but not interchangeably for both.

[8] Beck: *The Microscope.* Part II (London, 1924), pp. 67–69, and Johannsen: *Manual of Petrographic Methods* (1912), pp. 189, 190, give tables of comparative angular and numerical apertures, with limits of resolution for various wavelengths of light.

It is possible, with illumination of the proper degree of obliquity, nearly to *double* the resolving power of an objective. Since oblique illumination from one side only would give unsymmetrical shading to the object, light is generally made to converge upon the object from all directions symmetrically, by means of a condenser. The proper use of a suitable condenser is essential if objectives of high numerical aperture are to be utilized at their fullest efficiency (page 44).

Other Theories of Resolution. — Abbe's theory has been criticized because its experimental and mathematical foundation rests mainly on the optics of diffraction gratings.[9] However, the fine details of any structure may be considered as closely adjacent lines or points, which diffract light in a manner analogous to the adjacent rulings of a grating.

Helmholtz[10] and Rayleigh[11] have considered diffraction from a different point of view. If a point is imaged by a lens, it will appear as a point surrounded by concentric rings, of progressively diminishing intensity, the whole being known as a **diffraction pattern.** The diameter of this image pattern is governed by the aperture of the objective, the wavelength of light, and the refractive index of the medium between the point and the lens, all of which are comprised by the formula $\dfrac{1.2\,\lambda}{2\sin\dfrac{\text{A.A.}}{2}}$, which gives the diameter of the image point. Two adjacent image points will be seen as separate if the object points from which they originate are separated by the above amount. It will be noted that according to Abbe's theory the same factors govern resolution, the limit for axial illumination being $\dfrac{\lambda}{\sin\dfrac{\text{A.A.}}{2}}$; for oblique illumination it approaches $\dfrac{\lambda}{2\sin\dfrac{\text{A.A.}}{2}}$, which is essentially the same as Helmholtz' and Rayleigh's formula.

[9] Moore: *Jour. Roy. Micros. Soc.* (3) **48**, 133 (1928).

Gordon: *ibid.* (3) **49**, 123 (1929). The various theories are well reconciled by Rheinberg: *ibid.* (3) **49**, 132–42 (1929).

[10] *Pogg. Annalen.* Jubelband, **1874**

Gordon: On the Helmholtz Theory of the Microscope. *Jour. Roy. Micros. Soc.* **1903**, 381.

[11] *Phil. Mag.* **42**, 467 (1896); *Jour. Roy. Micros. Soc.* **1903**, 447, 474.

Importance of Resolving Power of Objectives. — The limit of resolution of an objective, as indicated numerically by the above formulas, is slightly greater than $\frac{\lambda}{2\,\text{N.A.}}$.[12] This theoretical limit is only attained with highly perfect lenses, proper illumination, and objects of good visibility. The use of objectives of high numerical aperture does not insure good resolution, though essential to it.

Resolving power is not merely a matter of ability to reveal the rulings of gratings; the chemist has to deal with even smaller structural details, in the study of powdered materials or alloys. Not only must the minute features of such specimens be revealed but there must be a true rendition of their size and shape. The faces and angles of tiny crystals must not be rounded or ill-defined; outlines of objects must not be surrounded by haloes or spurious diffraction bands. The boundaries of a specimen must be as fine and sharp as possible, and minute particles of transparent materials must appear as more than points of indefinite dimensions.[13] Measurements under the microscope are no more accurate than the resolving power of the objective. Many investigations of fine structures are carried on at the very limit of the resolving power of the microscope, and are often restricted seriously by its inflexibility.

Depth of Focus of Objectives. — Just as the ordinary camera will not simultaneously focus near and distant objects sharply, so microscope objectives will only image objects that lie approximately in one plane. Strictly speaking, the focus is perfect only for objects which are at exactly the proper distance from the objective; objects above or below this level are out of focus. Practically, if their difference of elevation is small enough, objects not in one plane may be in satisfactory focus simultaneously, due to the depth of focus of the objective.[14]

The property of **depth of focus**, or **penetrating power**, of an objective depends on the fact that the eye is incapable of recognizing small but finite deviations from a perfectly sharply focused image. Instead of an image point, a " circle of confusion " is observed if the focus is not exact, and this circle of confusion is larger the greater the aperture of the objective and the higher the magnification. The refractive index of the medium surrounding the object, and the power of accommodation and the acuteness of eyesight of the observer also affect the depth of focus somewhat.[15]

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[12] The number of lines per inch is sometimes given roughly as 100,000 N.A.

[13] Barnard: *Jour. Roy. Micros. Soc.* March, **1919**, p. 1, points out that objects below the limit of resolving power will appear larger than their true size.

[14] And also to the accommodating power of the eye, to some extent. See Carpenter: *The Microscope and Its Revelations* (1901) pp. 83–90.

The direct and unavoidable conflict between depth of focus and resolving power prevents any objective from possessing a maximum of both properties. Ordinarily, for low power microscopy objectives of high penetrating power are preferable, since they enable relatively thick objects to be seen in their entirety. For work at high magnifications, where resolving power is of the utmost importance, depth of focus is sacrificed to high numerical aperture. The depth of focus of a 1.40-N.A. objective, at a magnification of 1000 diameters, is less than 0.0005 mm., so that very precise focusing, by means of a highly accurate fine adjustment, is required.

Objects which are thicker than the depth of focus of the objective, or which differ in elevation by more than this value, are seen only by successive focusings, which bring a series of " optical sections " into view. Consequently lack of penetrating power seriously interferes with the usefulness of an objective for general microscopic work. For visual examinations the observer may be able to form a true concept of the structure of a specimen by studying its appearance with the focus at different levels, and combining his impressions. In photomicrography, however, one adjustment of the focus must be made and maintained for each exposure, and portions of the specimen may thus fail to appear well defined.

Objectives equipped with adjustable diaphragms to give variable numerical apertures are particularly valuable for studies of fairly thick specimens, where a compromise must be made between resolution and depth of focus (Fig. 5).[16] In using them the diaphragm may be closed just enough to give adequate penetrating power, with a sacrifice of no more resolving power than is absolutely necessary. This adjustment can be made for the particular portion of the specimen under observation, and is exceedingly convenient in photomicrography. Such increase in depth of focus also reduces the curvature of field (page 18) which is often objectionable in the photomicrography of flat specimens.

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[15] Marshall and Griffith: *Introduction to the Theory and Use of the Microscope* (London, 1928), p. 21.

[16] Objectives of this type are obtainable from the Spencer Lens Co. in focal lengths of 16, 8, 4, and 1.8 mm. Other manufacturers supply them in the higher powers only.

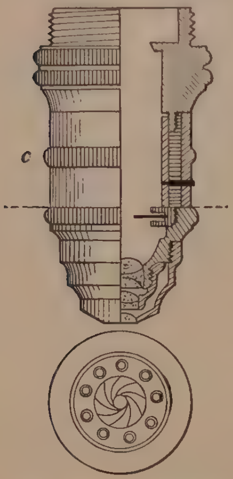

FIG. 5. Objective equipped with Iris Diaphragm (Leitz). The aperture is varied by rotating the collar *c*.

Penetrating power is not invariably an advantage in objectives, however, for it is sometimes useful to distinguish, by focusing, the relative elevations of different parts of an object (page 177), or to measure the differences in their levels (page 407). Objectives of the highest numerical aperture practicable, with correspondingly small depth of focus, are most suitable for such work.

Illuminating Power of Objectives. — The brightness of the microscopic image depends on the proportion of the total light emitted by the object which is included within the angular cone of the objective, and utilized to illuminate the image. Magnification and other factors being kept constant, the illuminating power varies as the square of the numerical aperture of the objective.

Light-Transmitting Power of Objectives. — Objectives of equal numerical apertures may give images of different brightness, depending on their light-transmitting power. This varies with the material, thickness, and number of surfaces of the various lenses in the combination, and also depends on whether the objective is "dry" or "immersion." Loss of light by internal reflections in the system is the chief factor which governs the light-transmitting ability of objectives.

Importance of Illuminating and Light-Transmitting Powers. — Both the above properties of objectives are of greatest importance at high magnifications, when the great areal enlargement causes a dimming of the brilliancy of the image. In photomicrography an unduly long exposure is necessitated, with increased risk of ill effects from vibration. It is therefore desirable that both the illuminating and light-transmitting powers of the objective should be maximum for such work.

Light-Uniting Properties of Objectives. — An objective possessing a high numerical aperture and capable of gathering a large angular cone of light from the object will still give a very imperfect image unless it is very carefully designed and constructed. All the diverging radiations from any point of the object which are included within the angular cone of the objective must be made to converge at a corresponding image point on the other side of the lens. They must arrive there together, not more than a small fraction of a wavelength out of phase, if resolution of fine structure is to be achieved. Equivalent optical paths are necessary, whether the rays pass through the edge or the center of the lens, and whatever their wavelength may be. An ordinary single lens, with spherically curved surfaces, does not satisfy the above requirements, and consequently gives images which are more or less diffuse, distorted, and surrounded by colored haloes. Lenses of high aperture exhibit these defects most seriously, and are worthless for purposes of resolution unless some correction of their aberrations is accomplished. These aberrations, which oppose the formation of a perfect image, fall into two classes: spherical, and chromatic.

Spherical Aberrations in Lenses. — A single lens, with surfaces which represent portions of spheres, is incapable of yielding a perfectly defined and undistorted image, even with monochromatic light. Its various defects, inherent in the spherical curvature of its surfaces, are spoken of as spherical aberrations. They may be lessened by reducing the aperture of the lens, at the expense of its resolving power, or by grinding the lens surfaces to nonspherical curves, a somewhat inaccurate process.

For lenses of high aperture, spherical aberrations are corrected by the use of several different lenses, of the proper radii of curvature, mounted together and acting as a unit to form a sharply defined image. The design of such combinations of lenses is a highly complicated branch of geometrical optics.

Spherical aberration manifests itself in all objectives to a greater or less degree, and affects the quality of the microscopic image very markedly if not minimized by proper correction.

A point on the axis of an uncorrected lens will be imaged at a greater distance by rays which pass through the center of the lens than by rays which pass through the edges. At no place will the point be imaged sharply, and a perfect focus will be unattainable

(A, Fig. 7). In order for this to be accomplished, the ratios between the sines of the angles which any two rays make with the axis must be the same on either side of the lens. This is spoken of as the **sine condition**, and it may be satisfied almost perfectly in a well designed objective, so that fine structures near the axis of the microscope (that is, in or near the center of the field) go in and out of focus sharply and are defined with satisfactory clearness. The correction for the sine condition must be made for a definite distance of image formation (usually that corresponding to a mechanical tube length of 160 mm., the common standard value).[17] The refractive index and thickness of any medium (such as a cover glass, or immersion liquid) between the lens and the object must also be taken into account.

Although spherical aberration may be practically eliminated for points near the axis of the microscope, it may still be apparent for points at some distance from the center of the field. Under these circumstances, it is impossible to obtain a sharp focus on any fine detail unless it lies in the center of the field — a very serious handicap in microscopic work, since the effective size of the field of view is thereby seriously restricted. Images of points appear unsymmetrical and more or less elongated, depending on the focus. This defect is known as **astigmatism**, and may be corrected by proper combination of the lenses. Even if this lack of symmetry in the image is eliminated, a point of perfectly sharp focus may still be lacking, just as when the sine condition is unfulfilled for axial points. The term **coma** is applied to this residual aberration.

Although it is possible to correct fairly well for the above aberrations, so that every point on a flat object is imaged sharply, these points are ordinarily not imaged in a plane, but instead their locus is a curved surface. Consequently the image of the entire field of the microscope does not coincide exactly with the focal plane of the eyepiece, and it is necessary to refocus the microscope in examining first the center and then the edge of the field. Such **curvature of field** is particularly objectionable in photomicrography, where a single position of focus must be selected for the exposure. It may be minimized considerably, but is very difficult to eliminate completely, especially in high-aperture objectives which are otherwise excellently corrected. The use of special eyepieces, or of amplifying lenses (page 38), helps to reduce the curvature of field somewhat. Unfortunately, curvature of field with objectives of high numerical aperture is particularly noticeable on account of the slight depth of focus which such objectives possess. An objective of low aperture, imperfectly corrected, may give a field which is flatter than that of an objective which possesses much greater resolving power and is otherwise very well corrected so as to yield a much superior image over a smaller field.

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[17] Objectives used on the larger metallographs are corrected by different makers for tube lengths ranging from 200 to 250 mm.

A further defect, closely related to curvature of field, is the **distortion** of the image at the edge of the field. It results in the object appearing slightly larger or smaller than it does in the center of the field. The nature of the distortion also varies with the type of eyepiece employed. Microscopic measurements are rendered inaccurate unless this aberration is taken into account. In addition, the shape of the object is distorted, so that angular measurements may be vitiated.

All the foregoing spherical aberrations may be corrected reasonably well by properly constructed combinations of lenses. Such a combination is spoken of as **aplanatic.** It may be composed of a single material, if strictly monochromatic light is used, as in the case of the " monochromats " of fused quartz, which are made for ultraviolet photomicrography. For microscopy with white light, however, a further set of corrections must be incorporated in the design.

Chromatic Aberrations in Lenses. — A simple or compound lens which consists of only one material will possess different focal lengths for light of different wavelengths, due to the dispersive power of the material. Images will be formed at different places, depending on the color of the light used; at no place will the image be sharp, with white light. Instead, it will be surrounded by colored haloes which interfere seriously with the observation of its true color. Such **chromatic aberration** may be lessened somewhat by decreasing the aperture of the lens, but much more perfectly by the use of compound lenses, the individual lenses of which consist of materials of different dispersive powers. For the correction of chromatic aberrations in an objective, two or more such substances (glasses or minerals) are utilized, and the perfection of the correction is limited by their several inherent dispersions. By a proper choice of optical glasses, of carefully selected dispersive powers, a lens may be made to give images at the same point, for light of two different wavelengths. For the remaining wavelengths of white light the images will be approximately at this point. Such a lens is said to be **achromatic**, and it will yield images free from prominent color haloes, though faint green and pink colored borders may be noticed in the image when the focus is shifted slightly. This slight **residual color** is not seriously objectionable for ordinary microscopic work.

For accurate observation of faint colorings, or for photomicrography, where an object is focused by visible light but photographed by the actinic ultraviolet portion of the illumination, it is necessary that the coincidence of images should be as perfect as possible. By the use of lenses of fluorite in combination with lenses of optical glasses, objectives may be corrected to produce coincident images for light of three different wavelengths. These **apochromatic** objectives give images exhibiting only an exceedingly faint blue or yellow residual color, and their actinic focus is the same as the visual focus. They also permit a better correction of spherical aberrations than is possible in achromatic lenses.

Objectives intermediate between achromats and apochromats in the perfection of their corrections are often called **semi-apochromatic**, or sometimes **fluorite** objectives if this mineral has been used in their construction.

Ideally, an objective corrected for three different wavelengths should exhibit none of the various spherical aberrations for these wavelengths. Practically, this is attained only in achromats for one wavelength, and in apochromats for two. The remaining aberrations are almost negligible, except near the edges of the field. Chromatically corrected objectives, particularly high-aperture achromats or apochromats, may give slightly different magnifications with light of different wavelengths, though they are well corrected for any single portion of the spectrum. This **chromatic difference of magnification** shows itself as color bands on the outer side of objects near the edge of the field. Special eyepieces, possessing a similar but opposite chromatic difference of magnification, are made to neutralize this aberration of the objective. They are called **compensating eyepieces** (page 33), and should always be used with apochromatic objectives.

Importance of Corrections of Objectives. — There is no advantage in objectives of high numerical aperture, if their corrections are so poor that the quality of the image suffers; the mere separation of details in the image is of little use if they are so hazy or so badly colored as to prevent accurate study.

The term **defining power** is sometimes used to refer to the ability of an objective to give sharp and colorless images. It is a measure of the perfection with which all the various aberrations have been corrected. Fortunately, in spite of the fact that all the aberrations of lenses tend to be much greater at high apertures, manufacturers have been able to correct objectives to a degree of perfection more or less commensurate with their resolving power and their magnifying power. These corrections are not expressed numerically, as are aperture and focal length, and they are indicated only by the words *achromat* or *apochromat* in catalogs or on objective mountings. The purchaser of an objective cannot afford to ignore their importance, however, since they may aid or limit his work quite as much as resolving power.

Magnifying Power of Objectives. — Contrary to the popular conception, the magnification of a microscope is less important than its resolving power, and since resolution is governed by the objective alone, while magnification depends on the eyepiece and image distance in addition, the light-gathering and light-uniting abilities of an objective deserve first consideration. Microscopes are used to reveal fine details, rather than to give enlarged images of what is already visible to the naked eye. It is true that these details must be rendered large enough to be seen in the image, but "empty" magnification which does not bring out additional minute structure is of little aid in the study of any object.

A well corrected objective of high magnifying power should have correspondingly high resolving power[18] in order to satisfy the above requirements. It is easier to make objectives of high magnifying power than it is to correct them properly and give them high numerical aperture. However, it is also easier to construct high aperture objectives of high power than it is to impart an equal

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[18] The term "optical index" has been proposed by Nelson (*Jour. Roy. Micros. Soc.* **1893**, p. 12) as a basis of comparison of objectives:

$$\text{O. I.} = 4 \times \text{N.A.} \times \text{focal length.}$$

The higher the optical index the greater the resolving power as compared with the magnifying power of the objective, and the more powerful the eyepiece which may be used with it. The optical indices of apochromats are generally higher than those of achromats of the same focal length.

numerical aperture to a low powered lens. As a consequence, the resolving powers of most objectives are more or less adequate to their magnifying powers. The corrections of high power objectives, on account of the high aperture demanded, are usually not quite as good as those of the lower powers.

Objectives are commonly designated by their focal lengths, to which their magnifying powers are inversely proportional. Magnification numbers (page 5) are also used as designations, by some makers.

Working Distance of Objectives. — The distance between the front lens of an objective and the object on which it is focused is called its working distance. The object is placed slightly outside the focal point of the objective, so that a real image may be formed. However, the focal length of the objective is usually considerably greater than its working distance, especially in lenses of high numerical aperture.[19] This is due to the fact that the focal length and image distances of a thick lens combination, such as an objective, are measured from its two **equivalent planes** (effective centers), instead of from the actual center as is the case with thin lenses (Fig. 1).[20] Part of the space between the front equivalent plane and the focal point is occupied by the lenses of the combination, thus reducing the working distance to less than the focal length. Obviously, the nearer the lower equivalent plane to the lower surface of the objective, the greater the working distance. By suitable combinations of lens elements it is possible to effect such a change in the location of the principal planes, though this generally involves a decrease in the quality of the corrections and in the numerical aperture of the objective.[21]

Importance of Working Distance. — For low power examinations, in which objectives of long focal length are employed, there is usually ample space between the lens and the preparation to permit focusing on the interior of reaction vessels, to allow space for necessary manipulations, and to avoid dangerous proximity of the objective to objects which are giving off heat or corrosive vapors. These requirements are met with difficulty by high power objectives of large numerical aperture and short focal length, so that their short working distance often renders them unsuited for certain chemical studies.

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[19] The working distance of a 2-mm., 1.40-N.A. apochromatic objective is only about 0.05 mm.

[20] The positions of the equivalent planes in several typical objectives are shown by Beck: *op. cit.* Part II, Fig. 38, p. 48. The locations of the focal planes of a number of objectives are given by Marshall and Griffith: *op. cit.* Fig. 15, p. 23.

[21] Four 4-mm. objectives of different grades made by the same manufacturer may have different working distances: apochromat, N.A. 0.95, working distance 0.18 mm.; semi-apochromat, N.A. 0.85, W.D. 0.34 mm.; achromat, N.A. 0.85, W.D. 0.3 mm.; achromat, N.A. 0.65, W.D. 0.6 mm.

Parfocalization of Objectives. — By properly regulating the positions of their upper equivalent planes, a series of objectives may be constructed which will require almost no refocusing when interchanged. They are said to be **parfocal** with respect to each other. This feature is a great convenience in changing from one power to another, but should be taken advantage of very cautiously when passing to a lens of shorter working distance.

The **size of field** of an objective depends on its magnifying power, and on the diameter of the diaphragm in the eyepiece at the plane of the real image.

Immersion Objectives. — The increased numerical aperture possible with immersion objectives (page 11) has led to their widespread employment for high resolutions. It should be borne in mind, however, that the nature of the preparation and the manner in which it is mounted may destroy all the advantage gained by their use.

It is necessary that the high refractive index be maintained throughout the entire space between the object and the objective, for the diffracted rays will be bent away from the perpendicular if they emerge into a less refractive medium, and will occupy a larger angular cone; moreover, the more oblique rays may be lost by total reflection, and may not emerge at all (*a*, Fig. 6). In this manner the full numerical aperture of the objective may fail to be utilized. The medium of *lowest* refractive index between the object and the objective is the limiting factor, and the numerical aperture of the objective will always be less than this value. An objective of N.A. 1.40 will have an *effective N.A.* of only 1.33 if used on an object mounted in water, even if the cover-glass and the immersion oil above it have a refractive index of 1.5.

The elimination of loss of light by total reflection at the surface of the cover-glass is not the sole basis of the superior resolving power of immersion objectives, as is implied in the usual diagrams. If this were true, increased resolution should be possible with uncovered objects, examined " dry." Actually, the " compression " of the diffracted rays by a highly refractive medium is the essential feature. (See page 11.)

Factors other than numerical aperture play a part in the superiority of immersion objectives. The efficiency of dry lenses of wide angular aperture is lowered because the rays most oblique to the axis of the lens, which are the ones most needed for resolution, are not perfectly transmitted by the surfaces of the cover-glass or the objective. At large angles of incidence a considerable portion of the light is lost by reflection, so that, although these rays are grasped by the objective, their intensity is low (*b*, Fig. 6). By the use of an immersion liquid having a refractive index much nearer to that of glass than has air, the transmission of these highly essential oblique rays is greatly increased, so that they can play their full part in resolution, and can also increase the brightness of the image.

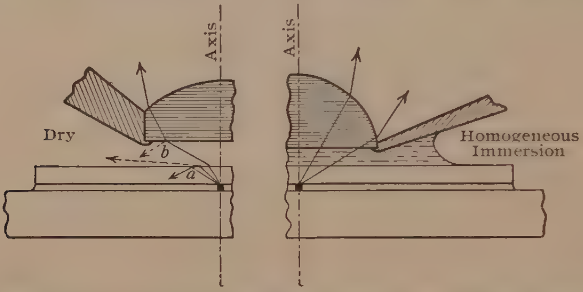

Fig. 6. Comparative "Light Grasping Powers" of Dry and Homogeneous Immersion Objectives.

Homogeneous Immersion Objectives. — If the immersion liquid has a refractive index and dispersion practically identical with that of the front lens of the objective, and of the cover-glass, if one is used, the system is spoken of as **optically homogeneous.** This involves the use of specially corrected **homogeneous immersion** objectives, which are worthless if used dry. Ordinarily, for glass lenses, thickened cedar wood oil ($n = 1.515$) is used as the immersion liquid. For fused quartz lenses a solution of glycerine ($n = 1.46\pm$) is used.[22]

With homogeneous immersion it is immaterial optically whether the object is covered by a cover-glass or not, since the light rays will follow the same path irrespective of their course being entirely through immersion liquid or partly through glass. The absence of reflection and refraction aids materially in the correction of these high-aperture lenses, and gives a more brilliant image than would be obtainable from a " dry " objective of similar aperture.

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[22] Martin: *Nature* **121**, 419 (1928).

Cover-Glass Corrections. — Objectives can be carefully corrected for a definite pair of object and image distances only if the thickness and refractive index of the medium (or media) between the objective and the object are taken into account. For objectives which are used for the study of uncovered objects (in metallography), this correction introduces no additional difficulties beyond those already mentioned. Homogeneous immersion objectives can likewise be corrected for the immersion liquid used, so that the cover-glass, of the same refractive index, will not affect the path of the rays, whatever its thickness.[23]

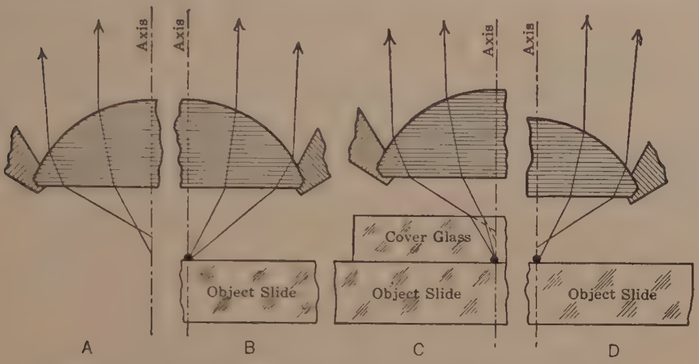

FIG. 7. Spherical Aberration and Cover-glass Correction.

A — Paths of rays toward a point in the image, through the front lens of an uncorrected objective.

B — Through an objective corrected for spherical aberration, and for use on uncovered objects.

C — Undercorrected objective for use with a cover-glass.

D — Spherical aberration when C is used on uncovered objects.

In systems which are not optically homogeneous the corrections are much less simple. A layer of glass between the object and the front lens of a dry objective has the effect of lessening the amount of the correction necessary for spherical aberration, so that if the objective is properly corrected for an uncovered object it will be over-corrected for a covered one, and will give a very poor image.

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[23] Some of the earliest homogeneous immersion objectives were so constructed for this reason, before their increased resolving power was known.

For this reason objectives of high aperture which are to be used for covered objects are purposely left slightly under-corrected for spherical aberration, the amount being calculated on the assumption that cover-glasses of approximately 0.18 mm. thickness will be used (Fig. 7). If thicker or thinner cover-glasses are employed, or if the object lies somewhat below the under surface of the cover-glass, the quality of the image will suffer. To provide for such variations in working conditions the better grades of high power dry objectives are equipped with graduated correction collars (Fig. 8) which, by changing the position of the lenses in the combination, adjust the spherical correction for various thicknesses of cover-glass.

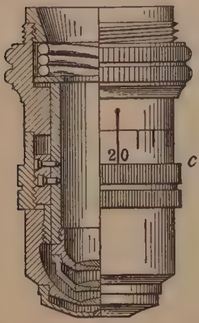

Fig. 8. Objective with Correction Collar.

By rotating the collar *c* the position of the two upper doublets is varied (Zeiss).

In the absence of a correction collar this adjustment may be made by altering the tube length of the microscope, increasing it beyond 160 mm. if the cover-glass is too thin, and decreasing it if too thick a cover-glass is used.

The adjustment of either the correction collar or the tube length, when the actual cover-glass thickness is not known, requires considerable practice. It is correct when the image of an isolated point in the object possesses the maximum sharpness when in exact focus, and develops identical blurring and halos whether the focus is slightly raised or lowered.[24] Ordinarily, adjustment for moderate variations in cover-glass thickness may be neglected for 8 mm. or lower power objectives, and powers lower than 16 mm. work equally well on covered or uncovered objects. Water immersion objectives do not function as homogeneous systems, and should be used with cover-glasses of standard thickness.

Construction of Objectives. — The variety of factors which enter into the calculations for correcting different aberrations render the designing of an objective a very complicated mathematical process. The actual construction and assembly of the component lens elements call for highly skilled workmanship and very careful testing. The individual lenses of the combination, certain of which are little more than a millimeter in diameter, must be shaped from perfectly uniform optical glasses. Their dimensions must be exact to a fraction of a wavelength of light. Some of them must be cemented together, their axes being made coincident. The various single, double, and triple lenses must be mounted so as to have a common axis, and must be exactly the proper distances apart. The mounting must not interfere with their optical functions, and must offer permanent protection. On the fulfillment of these requirements depends the perfection of the finished objective — a triumph of precision workmanship when one considers that a high power apochromatic objective may consist of ten lens elements, combined to give four or five compound lenses, and functioning as a unit to furnish a well defined image.

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[24] Beck: *The Microscope*, Part II (1929), p. 149.
Coles: *Critical Microscopy* (1922), p. 41.

Use and Care of Objectives. — Since the usefulness of an objective depends on the maintenance of its lenses uninjured and in their proper relative positions, great care must be taken to protect it from damage. A revolving nosepiece (page 52) will minimize the need for handling and the risk of mechanically injuring the objectives which are most frequently used.

In focusing downward while looking in the microscope there is great risk of passing the point of focus and bringing the lower lens of the objective forcibly into contact with the preparation, to the risk of injuring both. For this reason it is best to look *at* the objective while setting it to a position lower than the point of focus. Then, looking *in* the microscope, one can focus *upward* slowly until the image is sharp.

Preliminary study with low powered objectives, which cover large fields and have great depth of focus, will facilitate work with higher powers and give a truer concept of the general structure of the object. It is much quicker and easier to find and center details at low powers, and there is little risk of injuring such lenses, because of their long working distances.

Objectives should be kept protected from laboratory fumes, acids and alkalies, extreme heat and cold, or mechanical shock. They should be handled only with clean, dry fingers. Optical glass is much more susceptible to surface alteration than is resistant laboratory glassware, and the exposed lens surfaces of the microscope should be kept scrupulously clean. The lower lens of the objective should always be inspected after the examination of an uncovered liquid and if it has accidentally been wetted it should be carefully cleaned and dried at once. More than momentary contact with most liquids may injure both the glass and its delicate metal mounting. The fluids used with immersion objectives should be left on no longer than necessary. Immersion oil should not be allowed to become too thick, or its high viscosity may cause the cover-glass to move as the objective is focused.

Soft, long-fibered lens paper is used for cleaning the glass of optical instruments. For the removal of aqueous solutions it may be moistened with distilled water; for organic liquids, with xylene (alcohol may injure the lacquer or cement, and should not be used). Only paper which is clean and free from dust or grit is safe; sheets which are bound in book form are most easily kept from contamination, and may be torn out as needed. Paper which has been used or has been exposed to dust should never be employed in cleaning lenses.

When abrasive dust is present in the laboratory (for example, in metallographic work) the lenses should be blown upon (not " breathed upon ") and dusted with a soft brush, before being wiped with the lens paper.

The uppermost lens surface, at the back of the objective, should be protected by keeping an eyepiece always in the microscope. Dust on it may be seen by illuminating the field brilliantly, removing the eyepiece, and looking into the microscope draw-tube. A twist of lens paper about the end of a match stick will serve for cleaning purposes.

Only the front and back surfaces of the objective combination should be cleaned by the user. The interior lenses are well protected, and should remain free from dirt or fog. They should never be separated except by the manufacturer, lest the corrections of the objective be seriously impaired.

Choice of Objectives for Chemical Work. — The microscopical problems which chemists encounter are of such variety that no fixed assortment of objectives can be arbitrarily recommended. The user must choose his lenses on the basis of the work for which they will be used. In microscopic chemical analysis, long working distance and great depth of focus are important desiderata. Resolving power and perfect corrections become indispensable for magnifications much above 200 diameters, and particularly for work on pigments, fillers, and other fine powders. Flatness of field is noticeably important for photographic work, even at low powers, though a moderate curvature may be ignored in visual work. Correction "for uncovered objects" must be specified in the purchase of objectives for metallography, and the proper tube length must be adhered to, in case the same objectives are used on metallographs of different makes. Cover-glass "correction collars" are important on high power dry objectives. In making color distinctions, and particularly for photomicrography, apochromats are to be preferred, if used with eyepieces of the compensating type. If approximately monochromatic light is used, achromats may be satisfactory, and yield somewhat flatter fields than apochromats.

The focal lengths most commonly used for general work are: 32, 16, 8, 4 mm. for dry objectives; 2 mm. for immersion objectives. In such a series each objective has double the magnifying power and approximately double the resolving power of the preceding one. For special work, however, objectives of other powers and apertures may be desirable, and a study of the catalogs of the various manufacturers will give information as to the many different powers and types of lenses on the market.[25]

The actual testing of an objective with respect to all its numerical properties and its corrections for each kind of aberration requires considerable experience, particularly if lenses of high quality are being compared. The more glaring defects are obvious even to the beginner, but careful discrimination and much practice are required in judging the relative merits of good objectives of similar rating, from different makers.[26] Unless the user has trained his critical sense so as to be able to recognize the various residual aberrations which even good lenses exhibit, there is little justification for incurring the expense of purchasing highly corrected objectives and eyepieces, for their superiorities will never be noticed or utilized fully by a microscopist lacking in discrimination.

EYEPIECES

The **eyepiece** (or **ocular**) is an essential part of the optical system of the microscope as used for visual and for most photomicrographic work.[27] Its chief functions are:

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[25] Although catalogs list only the numerical properties of objectives and the degree of chromatic correction, a perusal of them will yield much useful information, particularly regarding special lenses which are not made by every manufacturer.

[26] Useful discussions of testing methods are given by Spitta: *Microscopy* (1920), Chapter V.

Beck: *The Microscope.* Part II (1924), Chapter VII.

See also Rogers: Test Objects for Metallography, *Jour. Roy. Micros. Soc.* **273**, 405 (1925).

[27] For photomicrography without an eyepiece, see p. 242.

1–To form a virtual image of the real image from the objective, in visual work.

2–To form a real image of the real image formed by the objective, in photomicrography.

3–To magnify the real image formed by the objective, in either of the above cases.

4–To image scales, crosshairs, or other objects located within the eyepiece.

As stated on page 5, the objective is focused so as to form its real image at or just inside the focal point of the eyepiece, in order to give a virtual image which may be observed by the eye (Fig. 1). In photomicrography and microprojection it is necessary that the objective be focused slightly upward from this position, so as to form its real image outside the focal point of the eyepiece (Fig. 104). Under these conditions, this real image is projected as a second re-inverted, real image on the photographic plate (page 244).

The magnifying power of the eyepiece is determined in each case by the ratio of image distance to object distance. Eyepieces are now commonly designated by their magnification numbers $\left(\frac{250}{f}\right)$ instead of by their focal lengths.

The angular apertures of the lenses of the eyepieces are small as compared with most objectives, but are adequate to resolve the detail of the real image. The angle at which the rays converge above the eyepiece is important where a cap nicol prism is used, since the obliquity of rays which can pass through the nicol is limited.

At the lower focal plane of the eyepiece is placed a diaphragm (F_2, Fig. 1), which defines the limits of the field of view (page 23). When micrometric scales, or crosshairs, are to be used they are placed at this level.

At the upper focal plane of the eyepiece parallel rays, originating at all points in the field included by the diaphragm opening, pass through a small circular area which is actually the image of the opening of the objective. This circular disk is called the **eyepoint** (or Ramsden disk) because the pupil of the eye must be placed at this level to receive light from all parts of the field (Figs. 1, 9, 10, 11). The height of the eyepoint depends on the focal length of the eyepiece, and the position of its upper equivalent plane. The diameter depends on the numerical aperture of the objective and the magnification of the microscope.[28]

Since the angular aperture of the lenses in eyepieces is relatively small, their aberrations are not so serious as are those of objectives. However, a poorly corrected eyepiece may limit the performance of a well corrected objective, particularly in photomicrography. Different types of eyepieces are manufactured, in order to give the necessary corrections and range of magnifications.

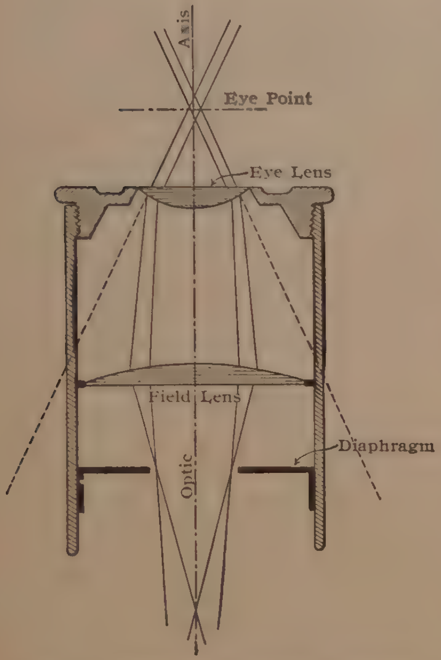

FIG. 9. Positive or Ramsden Eyepiece.

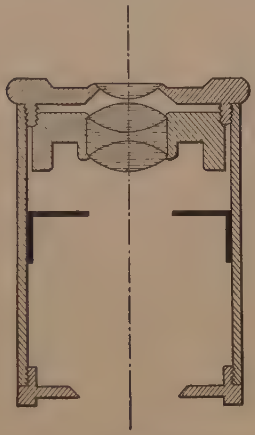

FIG. 10. Positive Eyepiece Corrected Lens.

Positive Eyepieces. — In a positive (or Ramsden) eyepiece a single lens combination is used. This may consist of two planoconvex lenses (Fig. 9) or, more commonly, of an achromatic doublet or triplet (Fig. 10). In either case, the combination functions as a unit, to give a more perfect image than that obtainable from a simple lens. As shown in the diagrams, the entire combination is above the plane in which the real image from the objective is formed.

Positive eyepieces are particularly appropriate for micrometry, for any distortion or other aberrations which they exhibit will affect the image and the scale equally. Furthermore, in the case of positive eyepieces the value of the micrometer scale divisions is directly proportional to the optical tube length.

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[28] The height and the diameter of the eyepoint may be measured by holding a ground glass above the eyepiece, the field being brightly illuminated.

Negative Eyepieces. — Eyepieces of another type consist of two lenses, one of which is below the image plane. Such a combination is called a negative (or Huygenian) eyepiece (Figs. 1, 11). The upper, or **eye lens,** functions as does an ordinary positive eyepiece. The lower or **field lens,** since it acts to modify the real image from the objective, may be considered as a part of the optical system of the latter. By its use the rays which go to form the real image are focused nearer to the objective, and the size of the image is reduced. This permits a larger area to be included in the field of view bounded by the diaphragm of the eyepiece, and gives a *brighter* but *smaller* image. The eye lens is so placed that its focus will lie in the plane of the image and of the diaphragm, just as in a positive eyepiece. Scales or crosshairs are located at this plane.

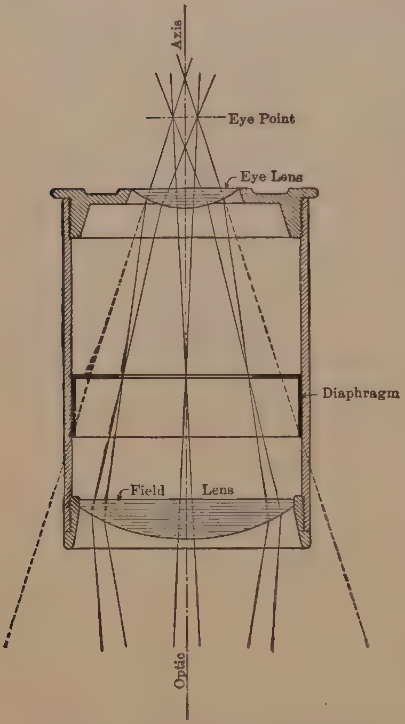

Fig. 11. Negative or Huygenian Eyepiece.

The eye and field lenses used in negative eyepieces are usually uncorrected, but their aberrations tend to neutralize each other, so that the combination is corrected well enough for ordinary work.[29] Greater freedom from aberrations is secured by the use of an eye lens consisting of an achromatic doublet or triplet.

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[29] A good discussion of the optics of eyepieces is given by Spitta: *Microscopy* (1920), Chapter VI.

Negative eyepieces of magnification greater than 12× are uncommon, for in the higher powers the necessary degree of correction is better obtained by the positive type of construction.

Corrections of Eyepieces. — Various degrees of correction are obtainable in either positive or negative eyepieces. Those which consist of two simple lenses only are called achromatic. They usually present a field of view which is bordered by blue at the edge of the diaphragm opening.

For more perfect correction, particularly the removal of residual chromatic aberrations, the image-forming lens consists of an achromatic doublet or triplet combination (Fig. 10). **Compensating eyepieces** are of this type, their chromatic aberration being calculated to neutralize the chromatic difference of magnification of the apochromatic objectives, with which they are used. Compensating eyepieces are not suitable for use with low power achromatic objectives, however, and may introduce some chromatic aberration into the image under these circumstances. The field of compensating eyepieces appears bordered with yellow.

Flat Field Eyepieces. — The combination of apochromatic objectives and compensating eyepieces usually gives fields which are far from flat. Special eyepieces have been designed to correct this defect. They are designated as " Hyperplane," " Periplan," " Planoscopic," by their respective makers. Such eyepieces give distinctly flatter fields than do compensating eyepieces, but their chromatic corrections are generally less perfect. They may be used with the higher power achromatic objectives without the introduction of chromatic aberrations in the final image, and in fact represent a compromise between the compensating and achromatic types of eyepieces.

Projection Eyepieces have the image-forming lens corrected to give a *real* image of maximum perfection. The position of this lens may be adjustable, so that the real image from the objective can always be formed at the proper tube length, and focused on the screen or photographic plate by movement of the upper lens only. (See page 245.)

Movable Eye Lenses are desirable on all eyepieces which contain crosshairs or micrometer scales. It is only by such an adjustable feature that the objects placed at the plane of the diaphragm may be focused simultaneously with the real image from the objective, irrespective of the near- or far-sightedness of the user. In photomicrographic work this adjustment is particularly important if the scale or crosshairs are to be projected as sharply as the object.

Parfocalization of Eyepieces. — A series of eyepieces of various powers is said to be parfocal if little or no refocusing of the microscope is required when they are interchanged. In positive eyepieces, this requires that their focal points and diaphragms shall all come at the same position in the draw-tube. In negative eyepieces, it is necessary that the images formed by the objective would all lie at the same level, if unmodified by the field lens.

Magnification and Resolving Power of Eyepieces. — Although the chief function of the objective is resolution, it is desirable that not too much of the magnification should be done by the eyepiece. Eyepieces of excessively short focal length are difficult to correct, and inconvenient to use. The " working distance " (height of eyepoint) is so short that the user's eye almost touches the eye lens. " Empty " magnification (page 39) is usually obtained with high power eyepieces, unless the objective has an exceptionally large numerical aperture for its focal length. The aberrations of the image from the objective are magnified and rendered unduly prominent by eyepieces of excessive magnifying power.[30]

Strictly speaking, in visual microscopy the eyepiece lenses should always be considered as acting jointly with the lens of the eye to give a real image on the retina (F_3, Fig. 1). The detail and the perfection of this image depend on the quality of both lenses, and on the aperture of the combination. If the illumination is so brilliant that the iris of the eye contracts, and the diameter of the pupil is less than that of the eyepoint, the resolving power of the eye will be decreased. If the numerical aperture of the objective is small and the magnification of the microscope excessive, the image of the objective opening at the eyepoint will be considerably smaller than the pupil of the eye, so that the full resolving power of the latter cannot be utilized. In addition, any dust on the eyepiece or on the eyeball becomes annoyingly prominent. Ordinarily the aperture and magnification of the microscope should be such that the eyepoint is not smaller than 1 mm. in diameter.[31]

For use with ordinary objectives in visual work, eyepieces of 10× to 15× magnification will reveal all the detail which is resolvable. Lower powered eyepieces may not magnify the image from the objective sufficiently for its finest details to be studied conveniently. In photographic work, the distance at which the eyepiece projects a real image governs the magnification, so that low power eyepieces are frequently used, with long camera extensions.

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[30] Eyepieces of exceptionally high magnifying powers (30×, 50×, 100×) are sometimes used in testing objectives.

[31] Wright (*op. cit.*, p. 135) discusses at length the eye as a part of the optical system of the microscope.

Use and Care of Eyepieces. — Low power eyepieces, with large fields, are preferable for preliminary studies of the general character of microscopic objects. High power eyepieces are used in the study of details, in conjunction with objectives of high aperture. Where depth of focus and long working distance necessitate the use of objectives of long focal length, the loss in magnification may be compensated by a high power eyepiece, though of course there is no compensation for loss of resolution.

In general the suggestions on page 27, made with respect to objectives, apply with equal force to eyepieces. However, it is permissible to take eyepieces apart for cleaning, if they are handled carefully. The inner surfaces of the lenses may be dusted with a soft brush, and wiped with lens paper. Usually dust on the upper surface of the field lens of negative eyepieces is most troublesome, and is noticeable as blurred spots which revolve when this lens is partly unscrewed. If the dust spots are not blurred but sharp, they are probably on the disk in the focal plane, on which cross lines or micrometer scales are engraved. This should be wiped very cautiously to avoid scratching or leaving fresh dust in place of that removed.[32]

The eye of the observer should (and will automatically) be placed at the eyepoint. The axis of the eyeball should be approximately parallel to the axis of the microscope. Spectacles should be worn, if they correct for astigmatism, but may be dispensed with if they are used only for near- or far-sightedness. In this case a slight refocusing of the microscope will permit the eye to form an image without special accommodation.

Both eyes should be kept open, in order to avoid eye strain and impaired vision. No attempt should be made to "see" the image at any particular distance such as the level of the table or of the stage. Instead, the attention should be concentrated on the field, and the eye relaxed completely. Focusing should be done wholly with the fine and coarse adjustments of the microscope and not with the muscles of accommodation of the eye. With a relaxed normal eye, and a properly focused microscope, the virtual image will be at "infinity," and a minimum of accommodation will be necessary. Under such conditions, eye strain is at a minimum, and if the illumination is properly adjusted little or no fatigue will result from several hours of microscopic work. As an aid in relaxing the eyes and relieving mental tension, it is well to look out of the window at distant objects frequently.

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[32] The microscopist should of course not attempt to wipe dust from cross-hairs which are made of actual filaments.

Choice of Eyepieces for Chemical Work. — Like the choice of objectives, the choice of eyepieces for chemical work depends on the type of examinations which are to be undertaken. For use with achromatic objectives ordinary achromatic eyepieces are satisfactory, though flat field eyepieces are preferable for high powers and for photomicrography. Apochromatic objectives require eyepieces of the compensating type. Projection eyepieces are used only for photography and microprojection. In high power photographic work where curvature of field is particularly objectionable, either flat field eyepieces or amplifiers (page 38) are desirable.

The eyepiece magnifications most commonly employed range between 5× and 20×, the lower powers being most used in photomicrography, and for micrometer scales.

In general, such combinations should be chosen as will yield magnifications in round numbers.[33]

Special Eyepieces. — In addition to eyepieces of the types described above, a number of special varieties are obtainable; their descriptions may be found in the manufacturer's catalogs, and their special uses in chemical work will be discussed in connection with the procedures for which they are best suited.

A very useful series of eyepieces has recently been developed, to obviate the difficulty which wearers of spectacles encounter in using ordinary eyepieces. They are constructed with exceptionally high eyepoints, and afford ample room for spectacles when the pupil of the eye is placed at the proper height.[34]

Demonstration Eyepieces divide the light from the objective, so that two images are formed, and two persons may observe the object simultaneously. They are equipped with a movable pointer which is located in the image plane.

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[33] See p. 255 for standard magnifications recommended for photomicrography.

[34] The "Telaugic" eyepieces of James Swift & Sons, and to a less degree the "Planoscopic" eyepieces of Spencer Lens Company possess this feature.

A focusing adjustment is usually provided, to compensate for differences in the eyes of the two observers. For instructional purposes, or for coöperative study of microscopic phenomena by microscopists and others, such instruments are invaluable.

By construction similar to that of demonstration eyepieces, **viewing eyepieces** have been made, chiefly for use in photomicrography. A semi-transparent reflector sends enough light to the eye of the observer to permit finding the object and focusing it. Simultaneously, the greater portion of the light is focused on the photographic plate or film. By this means the object may be observed, even during the exposure.[35] (See page 248.) The "Euscope" (Bausch & Lomb) is a device for projecting the microscopic image on a screen for visual observation. The user does not look in the eyepiece, but views this projected image as in ordinary vision. (See page 263.)

Comparison Eyepieces permit the simultaneous observation of the fields of two microscopes (Fig. 152). Some types take the place of the ordinary eyepieces. Others fit over them, and permit the use of eyepieces of any power, and hence a wider range of magnifications.

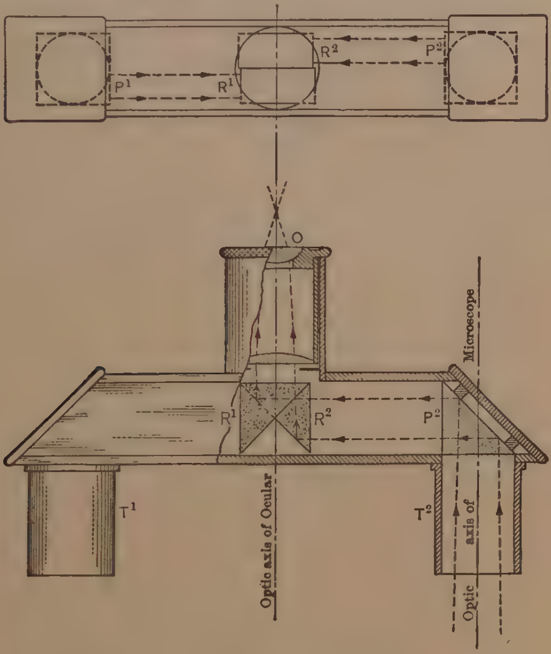

FIG. 12. Comparison Eyepiece (Bausch & Lomb).

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[35] Such instruments are manufactured by Zeiss and by Bausch & Lomb; similar features are incorporated into photomicrographic cameras by these makers and by Leitz.

The comparison eyepiece shown in Fig. 12 consists of a housing to which are attached the tubes T^1 and T^2, of the proper diameter and at such distance apart as to permit their being inserted into the draw-tubes of two microscopes placed side by side. Above each tube is mounted a 45° totally reflecting prism P^1, P^2, which reflect the image-forming rays from the two objectives to the 45° prisms R^1, R^2, situated just below the positive eyepiece O. On looking into the comparison eyepiece, the field is seen divided by a lateral line; this marks the boundary between the two halves of the fields of the respective microscopes. The fields of the two instruments should be separated in the eyepiece by as fine a boundary as possible.

Comparison eyepieces are indispensable when frequent comparisons must be made between unknown and known or standard preparations.[36]

Amplifying Lenses. — By the use of a concave lens in place of an eyepiece, the real image from the objective may be enlarged considerably (Fig. 13). Such "amplifiers," carefully corrected, have recently been placed on the market.[37] Since they form no virtual image, they cannot be used in visual microscopy, but are especially desirable for photomicrography on account of their ability to correct for the curvature of field which is always present with high power objectives. They are designed to give a colorless image with apochromats, and are an excellent substitute for compensating eyepieces. The magnification available is somewhat greater than that of a 10× eyepiece. "Homals" are made with different degrees of correction, depending on the type of objective with which they are to be used.

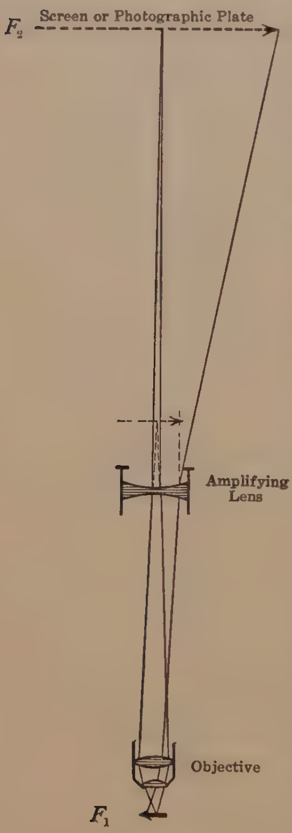

Fig. 13. Formation of a Real Image by an Objective and an Amplifying Lens.

Limit of Magnification and Resolving Power of the Microscope.

— If well corrected lenses are used, the magnifying power of the microscope should be *at least* that necessary to reveal the finest details resolvable by the objective. For the normal eye, this is equivalent to about 500 to 700 times the numerical aperture of the objective.[38] Since the details would be barely resolved by the eye at this magnification, it is preferable to place the limit of useful magnification for visual work somewhat higher — say 1000× N.A. For special purposes, such as micrometry and counting, higher magnifications may be desirable, though no new detail is revealed.

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[36] See also "Comparison Microscopes," p. 68.

[37] Boegehold and Köhler: *Zeits. wiss. Mikros.* **39**, 249 (1922). Manufactured by Zeiss under the name of "Homal." The "Ampliplan" lenses of Bausch & Lomb are similar in principle.

In photomicrography, the limit may be set still higher, for shorter wavelengths can be employed and the effective resolving power of the system thus increased.[39] By the use of ultraviolet light the maximum in resolution has been attained, and magnifications as high as 3000–4000× have been made justifiable.[40]

Photomicrographs which are viewed at some distance (such as pictures hung on the wall) or reproduced as halftones may require still higher magnifications, though this must be regarded as "empty" magnification.

It should be understood that there is no theoretical limit to the magnification possible with a microscope, if lenses of very short focal lengths and long image distances are used. "Super microscopes" have been constructed, in which a compound microscope is used instead of an eyepiece, to magnify the real image formed by the objective. Such excessive magnifications are of little value, and only accentuate the aberrations of the various lenses, so that the final image is of very poor quality.

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[38] Resolution in the eye is accomplished when adjacent details are imaged sufficiently far apart on the retina *not* to fall on adjacent nerve endings. If the magnification of the microscope is too low, such details will be imaged on adjacent nerve endings and will merge together. The limit of resolution of the microscope is to that of the naked eye as the ratio of their respective numerical apertures, and the magnification employed should be in similar or higher ratio:

$$\frac{\text{maximum N.A. of microscope}}{\text{minimum N.A. of eye}} = \frac{1.40}{0.002} = 700$$

or

$$\frac{\text{limit of resolution of eye}}{\text{limit of resolution of microscope}} = \frac{0.15 \text{ mm.}}{0.0002 \text{ mm.}} = 750.$$

[39] Discussions of high magnification and resolving power, particularly as applied to the photomicrography of metals by reflected light, are given in the following papers:

Lucas: *Trans. Am. Soc. Steel Treat.* **4**, 611 (1923); *Jour. Franklin Inst.* **201**, 177 (1926).
Guthrie: *Trans. Am. Soc. Steel Treat.* **7**, 4 (1925).
Mitchell: *Ibid.* **7**, 618 (1925).
Benedicks: *Metallographic Researches* (1926), p. 178, Chapter. V.

[40] Lucas: *loc. cit.*

The limit of resolving power of the microscope has already been stated, in terms of wavelength and numerical aperture (page 14). Practically, 0.0002 mm. ($0.2\,\mu$) may be considered the limit, for visual light,[41] and about 0.0001 mm. for ultraviolet light. Such limits are only obtainable with excellent lenses and objects of high visibility. They are not likely to be extended except by the use of light of still shorter wavelengths, and there are serious manipulative limitations to such developments. The greatest promise of extending the ability of the microscope to reveal fine detail lies in the perfection of methods of preparing and illuminating specimens so that the theoretical resolving power of the lens system can be fully utilized in all cases, instead of in only a few as at present.

The **limit of visibility** is a measure of the smallest particles which may be seen. It is less than the limit of resolution, and varies widely depending on the proximity of the particles, their color and refractive index, and particularly on whether bright field or dark field illumination is used. (See pages 94, 212.)

CONDENSERS

The objective and eyepiece comprise the image-forming part of the optical system of the microscope, but this can attain its maximum efficiency only when used in conjunction with an appropriate illuminating system, the most important part of which is the condenser.[42]

The chief functions of the condenser in low or medium power microscopy are:

1–To concentrate light upon the object, and increase the brilliancy of the image.

2–To furnish oblique or dark field illumination.

3–To project images of scales in the plane of the object.

In critical microscopy at high powers, the condenser has an additional and highly essential function:

4–To supply strongly convergent light to the object, as an aid in resolution.

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[41] Stoney: *Jour. Roy. Micros. Soc.* **1903**, p. 64.

[42] With vertical illumination the objective serves also as the condenser, and has similar functions (p. 116).

Concentration of Light by Condensers. — A condenser acts, as does any lens, to form a real image at or just outside its focal point. Usually the light source is so imaged, the condenser being placed so that the image comes approximately in the plane of the object (Fig. 14, A, F_1). The size of the image of the light source is governed by the ratio of its distance d to the distance at which the image is formed. The condenser thus forms a reduced image, roughly $\frac{\textit{focal length}}{d}$ as large as the light source. The reduced image is correspondingly more brilliant than the original light source.

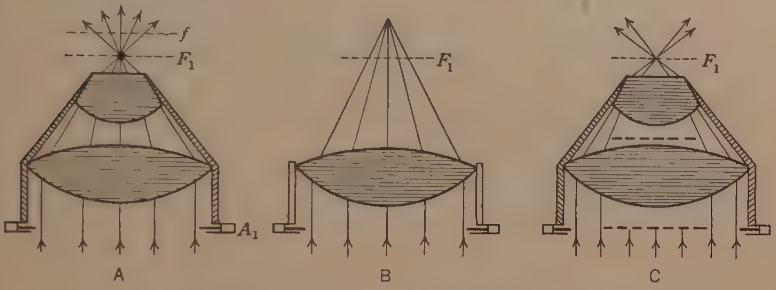

FIG. 14. Illumination by Means of the Substage Condenser.
A — Convergent illumination.
B — Illumination of a large field by the lower lens alone.
C — Dark Field (annular) illumination, with a "central stop."

The angular apertures of most condensers are fairly large, so that the light is strongly convergent at the point of focus. This convergence may be decreased by the use of an **iris diaphragm** A_1, with which most condensers or microscope stands are equipped, so that the intensity (and also the obliquity) of the illumination are adjustable. Some manufacturers graduate the iris diaphragm in terms of numerical aperture. The diaphragm should be located in or above the lower focal plane of the condenser, in order that it shall not act as a field diaphragm when the condenser is focused in certain positions.

The **focal length** of the condenser is important, for it determines the size of the image of the light source, and therefore the area illuminated. If the focal length is too short, the image of the light source will be too small to cover the field of the objective, and only the center will be illuminated. This defect is more or less common in high aperture condensers, on account of the practical difficulty of making them of long focal length. Fortunately, such condensers are most used with high-aperture objectives, which also have small fields. They require accurate centration, if their full efficiency is to be utilized.

In chemical work, however, condensers of long focal lengths and large fields together with fairly high apertures are necessary, because low or medium power objectives are generally used in preliminary studies, and for refractive index tests with oblique illumination.

Most condensers are made so that the top lens can be removed, the remainder of the lens combination having a longer focal length, and illuminating a larger area. Such **separable condensers** are best corrected for use in their entirety, but are also satisfactory for general illumination in low power microscopy. (Fig. 14, B.)

Long-focus **"spectacle lens" condensers** are also manufactured especially for low power work. They may be obtained in a number of different focal lengths.

The concave mirror may serve as a substitute for a low power condenser, to give convergent illumination. It should not be used in conjunction with condensers, however.

The **working distance** of a high-aperture condenser is usually much less than its focal length, the upper equivalent plane being considerably below the uppermost lens surface. Frequently the condenser must almost touch the lower surface of the object slide in order to focus on the specimen mounted upon its upper surface. This is usually not objectionable, unless exceptionally thick slides are used. If the working distances of the condenser, measured in glass, is less than about 1.5 mm., selected thin slides are required.

Oblique Illumination by Condensers. — The cone of rays from the condenser may be utilized in its entirety as **symmetrically convergent illumination.** By closing the diaphragm its angle may be decreased, and a narrow beam substantially parallel to the axis of the microscope may be obtained. This may also be accomplished by lowering the condenser. (Fig. 14, A, *f*.) If only one side of the condenser aperture is left open, the rest being closed by an opaque screen, **unilateral oblique illumination** is obtained. An excenterable iris diaphragm, a piece of cardboard in the ring attached to the condenser mounting, or even the finger held over one side of the fully opened diaphragm (Fig. 15) will permit easy changing from symmetrical to oblique illumination, in testing for refractive index (page 369), or as an aid in the interpretation of appearances (page 78).

The degree of obliquity is determined by the angular aperture of the condenser, and also by the extent to which the axial rays are stopped out. If the angular aperture of the condenser is much greater than that of the objective used, it is possible to render the illumination so highly oblique that no direct rays enter the microscope. This is rarely done in practice, but highly oblique illumination from all azimuths is frequently employed. By the use of an opaque disk (central stop) placed in the center of the diaphragm opening of the condenser, an annular aperture is left through which a hollow cone of rays passes to the object (Fig. 14, D). The size of the stop must be such that no direct rays will be included within the angular cone of the objective. Under these conditions **annular or dark field illumination** is obtained, and the surfaces of objects appear self luminous against a dark background.

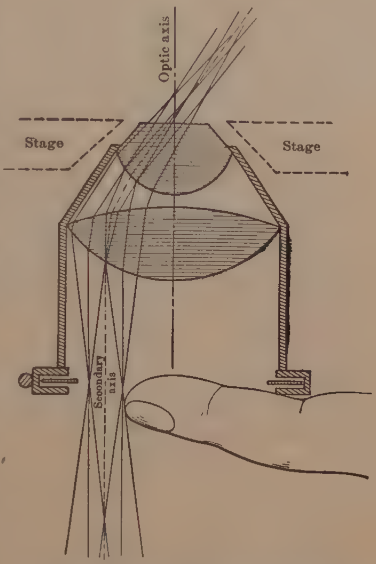

FIG. 15. Oblique Illumination by Screening One Side of the Aperture of the Substage Condenser.

Separable condensers are manufactured in which the top lens is replaceable by a similar lens with central stop, or the central stop may be inserted just beneath the top lens (Fig. 14, C). Combination condensers are also made, to permit rapid transition from dark field to bright field illumination. In general, only very well corrected condensers, of high numerical aperture, are suitable for dark field illumination at high powers, and special dark field illuminators (page 87) are more satisfactory.

Projection of Images by Condensers. — The projection by the condenser of an image of the light source in the plane of the preparation has been mentioned above. In a similar manner other objects may be imaged in the plane of the preparation. Diaphragms to regulate the area illuminated (pages 98, 126), special scales for micrometry (page 403), or color standards for comparison (page 189), may thus be superposed upon the image of the object. For such purposes a well corrected condenser is of course essential.

By diaphragming the condenser strongly, its aberrations are minimized, and the projected image may be good enough so that it may actually be used for viewing objects outside the microscope, as a long- or short-range telescope of low resolving power. A "Telemicroscope" has been constructed on this principle, and a "Telopic" lens is manufactured for a similar purpose.[43]

Strongly Convergent Illumination by Condensers. — The increase in resolving power of the microscope, obtainable by oblique illumination, has already been discussed (page 12). To realize the doubling of the resolving power which is theoretically possible, it is necessary that the obliquity of the illuminating rays should be the same as that of the most divergent rays which can pass through the objective. In other words, the numerical aperture of the condenser should equal that of the objective. Condensers of numerical apertures as high as 1.40 are required, if the limit of microscopic resolution is to be reached.

The cone of rays from the condenser should converge exactly on the object, if its full aperture is to be utilized. The necessary adjustment is made by focusing the image of the light source in the plane of the object. This **"critical" illumination** should be employed in all work at high powers, where fine details are sought.[44]

Corrections of Condensers. — For very low power work the condenser may consist of a single uncorrected converging lens, such as a "spectacle lens" condenser. Where more strongly convergent illumination is required the **Abbe condenser** is used. In its simplest form the Abbe condenser consists of two lenses (Fig. 14, A), and possesses marked spherical and chromatic aberrations. It serves to supply convergent or oblique light, but is unsuitable for critical work, since not all its rays focus in one plane. By the combination of more lenses, the Abbe condenser may be corrected more perfectly, but its spherical aberrations are serious, and it is reasonably aplanatic for only a part of its full aperture.[45]

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[43] James Swift & Sons, London.

[44] Further discussion of methods of critical illumination is given on pp. 97, 118.

Condensers appropriate for critical microscopy are carefully corrected, especially for spherical aberration. These **aplanatic condensers** (Fig. 16), are usually practically achromatic also, and give a relatively perfect convergence of light even at their full apertures. Their focal lengths and working distances are usually less than those of ordinary Abbe condensers. If the top lens is separable (as at A, Fig. 16), this is not a serious objection, for the remainder of the combination is adequate for low power work.

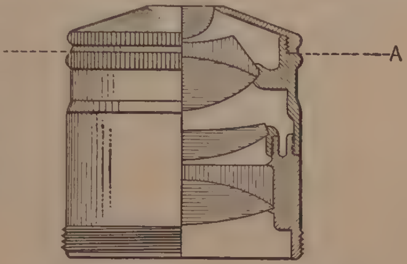

FIG. 16. Aplanatic Achromatic Condenser, N. A. 1.40 (Leitz).

The top lens is separable at A, for the illumination of larger areas.

Immersion Condensers. — As in the case of immersion objectives, a highly refractive medium must fill the space between the object and the condenser, if numerical apertures greater than 1.0 are to be attained. The refractive index of the immersion liquid limits the *effective* numerical aperture of the condenser; a 1.40-N.A. condenser will yield a 1.33-N.A. cone of light, if "immersed" with water, and a 1.0-N.A. cone of light if used dry. The rays outside these angles are totally internally reflected, and do not emerge from the condenser.

The *lowest* refractive index between object and condenser governs the cone of rays which comes to the object. Objects mounted "dry," and not in optical contact with the slide, can only receive a cone of light of 1.0 N.A. The excess is lost by total reflection at the upper surface of the slide. Strictly speaking, high aperture condensers should be used only with homogeneous immersion oil ($n = 1.515$) if their corrections are to be utilized perfectly. Actually the layer of liquid between condenser and object slide is so thin that little aberration is introduced if the system is not optically homogeneous. However, the limiting factors mentioned in the preceding paragraph should be borne in mind, if less refractive liquids (such as water) are used for the sake of convenience, or if the condenser is used dry. If the condenser is diaphragmed down, instead of being used "wide open," its *working* aperture may not require the use of an immersion liquid.

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[45] The importance of the *aplanatic* cone of the condenser, as a measure of its usefulness, is discussed by Barnard and Welch: *Practical Photomicrography* (London, 1925), pp. 47–54. See also Marshall and Griffith: *op. cit.* Plate II, p. 28.

Condensers of high apertures are corrected for use with slides of a definite thickness (analogous to cover glass correction of objectives), but if homogeneous immersion is used slides of any thickness less than the working distance of the condenser (measured in glass) may be used. Homogeneous immersion also increases the illuminating power of the condenser, by minimizing the loss of a portion of the light from reflection as it strikes the surface of the top lens, or of the slide, obliquely.

Objectives as Condensers. — Largely in order to take advantage of their superior corrections, objectives have been used as condensers. They are wholly unnecessary for low power work, while for high power work well corrected condensers are much cheaper. The working distances of high-aperture objectives are so short that they will not focus the light through an ordinary slide, and the object must be mounted between two cover-glasses.

In the examination of opaque objects by means of vertical illuminators, the light enters the back of the objective, and is focused by it on the surface of the object. The objective thus serves a dual purpose, as condenser and as objective. The foregoing discussion of principles of condensers as used for illumination by transmitted light is applicable to illumination by reflected light, as discussed on page 116.

Use and Care of Condensers. — In order that the condenser shall fulfill its various functions, it is necessary that it shall be used in a manner appropriate to the character of the work. In low power microscopy the ordinary Abbe condenser, approximately in focus for convergent or oblique illumination, and diaphragmed and lowered for axial illumination, requires no particular care in its adjustment. The plane side of the mirror should be used, since condensers are not constructed to deal with convergent rays. The diaphragm should be adjusted by trial to give maximum visibility and contrast in the image.

In critical microscopy, the use of high-aperture condensers requires more careful manipulation.

The condenser must be accurately centered with respect to the axis of the objective, in order that its full aperture may be effective and that it may give symmetrical and uniform illumination over all parts of the field. Either the substage, the condenser mounting, or the nosepiece which holds the objective should possess an adjustment for centration. The condenser may be considered centered when the small opening of a diaphragm placed concentric with its back lens is seen at the *center* of the back aperture of the objective, as observed after removal of the eyepiece.

The adjustment of the condenser so that it fills the aperture of the objective with light is best carried out similarly, any defects in the illumination being noticeable at the back of the objective. The relative opening of the iris diaphragm may also be observed there. The diaphragm opening should never be greater than is sufficient to supply illumination in a cone equal to that of the objective, or the object will be veiled by the excess light and will present little contrast. Ordinarily, the aperture of the condenser, as seen by looking in the body tube of the microscope,[46] should be about two-thirds that of the objective, though it may be necessary to close the diaphragm still further in the study of objects of poor visibility.

The size of the light source affects the quality of illumination obtained by the condenser. As pointed out by Beck, and by Wright[47] the area illuminated should be no greater than the field under examination, or "glare" and loss of contrast will result. The size of the image of the light source may be regulated somewhat by varying its distance from the condenser, but some means of regulating the size of the light source itself is desirable. Light from the sky cannot conveniently be so regulated, but artificial light sources in combination with auxiliary condensers (pages 96, 123) give an easy means of obtaining the best possible critical illumination with the minimum of glare.

Condensers should be cared for as are the other lenses of the microscope, being kept clean and free from corrosive materials. Immersion liquid should be used sparingly and not allowed to run down over the mount of the condenser; it should be completely removed immediately after use.

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[46] If the condenser is provided with an iris diaphragm graduated in numerical aperture, the above procedure may be employed to determine the numerical aperture of the objective.

[47] *Jour. Roy. Micros. Soc.* **261**, 399 (1922).
Principles of Microscopy (1906), Chapter XV.

Dust and scratches on the top surface of the combination are particularly objectionable, for they may be visible in the field of the microscope.

Choice of Condensers for Chemical Work. — The principal features of various types of condensers have already been discussed, and should serve as criteria for their selection. The quality of the condenser should correspond to that of the rest of the optical system of the microscope. Abbe condensers are suitable for use with achromatic objectives, but for critical work at high powers aplanatic condensers should be chosen.

Condensers are usually designated only in terms of their numerical apertures and aplanatic and achromatic character. Their working distances and the size of field illuminated (as governed by the focal length) are not very commonly given, and usually have to be determined experimentally.

The focus and obliquity of the illuminating cone supplied by various types of condensers may be observed by means of a cube of uranium glass placed above the top lens, and in immersion contact with it if necessary.

DIAPHRAGMS IN THE MICROSCOPE

In addition to the lenses of the optical system of the microscope, the various diaphragms are important, for they regulate the apertures of the various lenses and the size of the fields imaged by them.

Aperture diaphragms are placed so as to limit the cone of rays which is transmitted by their respective lenses.

Field diaphragms are placed so as to be imaged coincident with the image of the object, and to limit the area visible.

Reducing the opening of an aperture diaphragm reduces the illumination uniformly over the entire field. Although the light passes through a restricted opening, it converges at each point of the image just as when a larger aperture is available, and no decrease in the size of the illuminated field results.

Reducing the opening of a field diaphragm decreases the intensity of the light uniformly over the entire aperture. The full opening is utilized by the cone of rays which converges in each image point, but less light is required since the area of the illuminated field is decreased. The brightness of the illuminated portion is not reduced, though its area is smaller.

The above characteristics may be utilized as tests to aid in recognizing whether a given diaphragm functions to govern aperture or field. Both kinds of diaphragms are essential in the optical system, but diaphragms located in intermediate positions affect both field and aperture, and do not permit independent regulation of these two factors. Unfortunately, the illuminating systems of some metallographic microscopes possess diaphragms of this dubious character.[48]

From Fig. 1 it will be seen that A_1, A_2, A_3 mark the planes of aperture diaphragms in the condenser, objective, and eyepiece (and eye) respectively. Although each of these apertures may be regulated independently, their images are superposed. The aperture diaphragm of the condenser, in its lower focal plane, is imaged in the upper focal plane of the objective, and both of these are imaged in the upper focal plane of the eyepiece, at the eyepoint where the pupil of the eye is placed. Their relative magnitudes may be observed at the back of the objective, as already described, or by examining the eyepoint with a low power magnifier.

The possible locations of field diaphragms are indicated by F_1 and F_2 in Fig. 1. Diaphragms in the plane of the object F_1 are used only in low power microscopy without a condenser. The area of the light source, imaged in this plane, functions as a field diaphragm.

The eyepiece diaphragm, F_2, defines the size of field sharply, and cuts off the portion of the image in which the aberrations are worst.

Other field and aperture diaphragms, belonging to the illuminating system and external to the microscope proper, are discussed in connection with illumination (pages 84, 97), ultramicroscopy, (page 214), photomicrography (page 247), and metallographs (page 127). Diaphragms are also of considerable importance in conoscopic observation of the interference figures of crystals (page 288).[49]

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[48] Various screens may be located in the optical system to cut off internal reflections of stray light, but their openings are always large enough to permit the passage of all image-forming rays, so that they do not act as either field or aperture diaphragms.

It should be borne in mind that the various diaphragms of of an optical system, though imaged in identical planes, are not optically equivalent. The aperture diaphragm of the condenser governs obliquity of illumination and contrast; that of the objective, its resolving power and depth of focus; the eyepoint, the quality of the retinal image. The area of the light source, acting as a field diaphragm, governs uniformity of illumination and " glare " from stray light; the eyepiece diaphragm, the area of the field only. Each of the several aperture or field diaphragms has its particular function which cannot be adequately filled by any other diaphragm.

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[49] In the various diagrams of the optical systems of apparatus possessing field or aperture diaphragms, the same designations have been used in order to facilitate comparisons and to emphasize analogies. F, F_1, F_2 refer to field diaphragms or to the location of objects or real images, and A, A_1, A_2 to aperture diaphragms.

CHAPTER II

MICROSCOPES FOR USE IN CHEMICAL LABORATORIES

The optical systems of all compound microscopes are essentially alike, in that they comprise an objective, an eyepiece, and, usually, a condenser. The mechanical design and construction of the instruments vary greatly with different makers.

GENERAL MECHANICAL FEATURES OF MICROSCOPES

The **stand** of any microscope serves primarily to support the object and the various parts of the optical system in proper relation to each other. Its essential features are:[1]

1–The body-tube, to carry the objective and the eyepiece.

2–The focusing apparatus, to move the lens system.

3–The stage, on which the specimen is placed.

4–The substage, in which the illuminating apparatus is mounted.

The **body-tube** should be provided with a graduated telescoping extension, the **draw-tube**, in order that the **tube length** may be varied (page 5). If the range is from about 150 mm. to 180 mm., it will allow sufficient adjustment for micrometry (page 398), will permit the use of objectives of different corrections (page 18), will enable the tube to be shortened if a vertical illuminator is attached (page 123), and will serve as a means of correcting for variations in cover-glass thickness (page 26). The user should ascertain whether the tube length indicated by the graduations includes the revolving nosepiece, the vertical illuminator, or other attachments which may be fastened to the tube at the time of purchase.

The draw-tube should not move too freely, in order to avoid creeping from the weight of any attachments such as filar micrometers, spectroscopic eyepieces, or cameras, which may be placed upon it.

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[1] A number of points in connection with the design and construction of stands for general use are given in *The Microscope, A Symposium.* Edited by F. S. Spiers (Chas. Griffin Co., London, 1920). See also the descriptions of microscopes by Krause: *Enzyclopädie der Mikroskopischen Technik,* II Band (Berlin, 1926), pp. 1494–1512.

The dimensions of the body-tube are almost universally standardized, the opening to carry the objective being threaded according to the screw gage of the Royal Microscopical Society.[2] The standard inside diameter of the upper end of the draw-tube is likewise fixed at 23.2 mm., for use with ordinary eyepieces. If eyepieces of large diameter are to be used, the diameter of both the body-tube and the draw-tube are greater, and an adapter should always be provided with monocular microscopes, to permit the use of eyepieces of standard diameter.

Most manufacturers supply an extra large body-tube with their more expensive stands for photomicrography, but this wide tube serves no useful purpose unless the *entire upper end* including the sleeve of the draw-tube is removable for low power photomicrography without an eyepiece (page 242) and unless there is also supplied with the instrument a special adapter to connect it with the camera, when the draw-tube is removed.

Objective changers. — In general microscopic work frequent changes of objectives are necessary, to vary magnification or working distance, or obtain better resolution, etc. To avoid the inconvenience and loss of time from unscrewing one objective and inserting another, various devices are attachable to the body-tube. Of these, the **revolving nosepiece** is preferable for chemical work; it should be of the dust-proof construction shown in Fig. 17. Three or four objectives are thus permanently attached to the microscope, and are instantly available with a minimum of handling. If they are parfocal, interchange is possible with little or no refocusing. The centration of the fields of the different objectives, when used on a well made revolving nosepiece, is usually perfect enough for all ordinary work, and the ease of manipulation outweighs the slight re-centering of the object which may be necessary when shifting to high powers.[3]

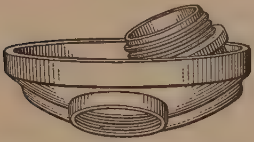
FIG. 17. Dust-proof Revolving Nosepiece.

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[2] If it is easily accessible, the lower end of the draw-tube should be similarly threaded, for the insertion of objectives of great working distance.

[3] If the objectives are carefully adjusted on the nosepiece by means of paper washers, practically perfect centration and parfocalization may be obtained. Revolving nosepieces with individually centerable objective openings are supplied on the polarizing microscopes of Zeiss-Winkel.

The clutch objective changer shown in Fig. 22C is superior to revolving nosepieces as regards centration but requires that the microscope shall be thrown completely out of focus whenever objectives are interchanged.

Maximum precision of centration, such as is sometimes necessary in photomicrographic work at high magnifications, is made possible by the use of **sliding objective changers** of the type shown in Fig. 18. The plate *a* is screwed to the lower end of the body-tube, and serves to receive the individual slides *b*, to which the different objectives are permanently attached and centered by the screws S, S′. A special case for holding the objectives while on their slides is desirable for use with sliding objective changers.

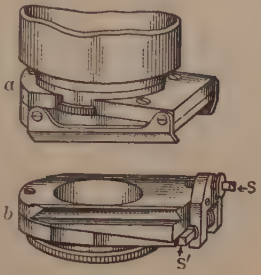

FIG. 18. Sliding Objective Changer, with Centering Screws (Zeiss).

The **focusing apparatus** ordinarily consists of a rack and pinion **coarse adjustment**, and some form of fine adjustment. Since the coarse adjustment is often used for fairly accurate focusing, it should operate smoothly and without lost motion. Its bearings should be adjustable, to take up wear; the friction should be sufficiently great to prevent downward creep from the weight of any accessories which may be attached to the draw-tube. The upper limit of the range of the coarse adjustment should be as high as possible, to permit the examination of thick objects on the stage by means of objectives of long working distance.

The **fine adjustment** is one of the most important mechanical features of any microscope used for medium or high magnifications. It must be so perfectly made that the position of a high power objective may be controlled with an accuracy comparable with its depth of focus — in other words, to less than 0.001 mm. Lost motion and "lag" are particularly objectionable, and should not be detectable in a well made movement. If they develop with use, the instrument should be returned to the maker for cleaning and readjustment. Rugged construction is to be desired but the fine adjustment should be operated carefully, and never forced if it has been turned to the limit of its range. For most work a moderately quick motion screw on the fine adjustment is preferable to one which requires considerable movement to focus an objective of medium power. Very sensitive fine adjustments are necessary for work at high magnifications, however. The head of the fine adjustment should be graduated,[4] to serve as a micrometer for vertical measurement (page 407).

The **stage** should be wide, with plenty of space beneath the handle arm for large specimens. Rectangular stages, although popular for biological microscopes, are not as satisfactory for general work as are rotating stages. The circumference of the rotating stage should be graduated in degrees. Some means of locking the stage against rotation, when using a mechanical stage, is a desirable adjunct. Centering screws should be provided (page 273); they are also convenient for limited movement, as an occasional substitute for a mechanical stage.

The central opening in the stage should be at least 25 mm. in diameter, to permit condensers having large mountings to be raised level with the upper surface of the stage. If the entire stage (and its bearings) is easily removable, hot stages or other accessories can be inserted in its place, and cleaning and lubrication are facilitated.

The **substage**, in practically all modern microscopes, consists of a holder for the condenser or other illuminating apparatus. This holder is movable vertically by a convenient quick-acting screw, or a rack and pinion. If the opening of the substage is a simple ring, its inside diameter should be 38.8 mm., to permit the interchange of standard condensers.[5] The movement of the substage should be smooth and accurate, so that high-aperture condensers or dark field illuminators may be focused with precision. Some means of centering the substage should be provided, if such accessories are to be used.

The mirror should swing to one side, if possible, and should be reversible, plane and concave. Lowering the substage should not disturb its position.

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[4] The value of one division of the graduations is seldom marked on the stand, but can be found in the maker's catalog.

[5] The microscopes of Zeiss have a somewhat smaller substage ring, so that condensers by other manufacturers cannot be used without special adaptation. Substages in which the illuminating apparatus is carried in a fork or slide may not allow the use of standard condensers unless constructed to meet this requirement. The condensers made by E. Leitz are of slightly larger diameter than the standard.

The frame of the microscope should be of roomy dimensions, and easily handled, with a low center of gravity. The instrument should be perfectly steady even when inclined to the horizontal position.

The finish should be smooth, black and chemically resistant. Polished brass parts should be as few as possible, to minimize annoying reflections and to insure a more permanent finish.

MICROSCOPES FOR CHEMICAL USE

The problems in which the chemist is called upon to use the microscope are so diverse in their nature, the materials to be examined are of such differing size, form, and structure, the examinations involve such a wide range of magnification and illumination, and the properties which must be determined are so numerous, that it is safe to say that no single instrument will meet all requirements and all conditions. Before deciding upon any given style or model of instrument, the intending purchaser should therefore consider carefully the kind of work for which his instrument will most frequently be utilized.

Until quite recently the microscopes available have been designed primarily for biological work, and are of limited application in the enormous variety of investigations which arise in chemistry and technology. As time goes on, however, instruments of more universal character are being developed, to extend the capabilities of the microscope beyond that of a mere magnifier. Highly specialized types of apparatus are also being manufactured to meet the need for microscopes designed primarily for the rapid and accurate examination of certain classes of materials such as crystals and metals.

In addition to the requirements already mentioned as applicable to all microscopes, the chemist should be guided in his selection of an instrument by a consideration of which model will permit the maximum ease and completeness of **observations with polarized light.**

The essential features of a polarizing microscope are discussed in detail in Chapter IX. Since their incorporation need not decrease the usefulness of the microscope for ordinary work, any instrument selected by the chemist should possess as many of them as possible, without loss of simplicity or sacrifice of the basic requirements which have been discussed.

To permit the fullest use of nicol prisms they should be attachable to the microscope in definite positions, crossed with respect to each other. A stud on the mounting of the polarizer should engage a notch in the substage ring or fork. The draw-tube should be "keyed" to move up and down without rotation. The collar, which is mounted on the upper end of the draw-tube and carries the analyzer, should engage a notch or stud so that its position will always be the same with respect to the microscope as a whole. The nicol prisms may thus be removed and replaced in the proper "crossed" position. A notch in the upper edge of the draw-tube is also necessary, to engage a stud on the eyepiece. The crosshairs in the eyepiece must represent the planes of vibration of the two nicol prisms, when crossed.

The **general specifications for a simple chemical microscope** may be summarized as follows:

1–Stand. Substantial, roomy, adaptable. To take objectives, eyepieces, and condensers of standard dimensions. Finished entirely in black.

2–Body-tube. Draw-tube keyed, with stud for collar to carry analyzer and notch for stud on eyepiece. Graduations to include nosepiece.

3–Coarse adjustment. Maximum range possible.

4–Fine adjustment of moderate sensitivity. Graduated in divisions and revolutions, with value indicated.

5–Revolving nosepieces. For three objectives. Selected for good centration.

6–Stage. Rotating, of maximum diameter. Graduated in degrees. Centerable by two screws, and readily removable. Lock screw, to prevent rotation. Provision for attaching a mechanical stage.

7–Substage. Focusing. Accurately centered, or centerable. Of standard diameter. Notch for stud of polarizer mounting.

8–Polarizer. Nicol prism, rotatable, with pointer and click at zero setting. Stud in mounting, to engage notch in substage. Plane of vibration indicated by the vertical crosshair of the eyepiece.

9–Analyzer. Glan-Thompson prism, rotatable, graduated. Plane of vibration indicated on mounting. Carried in collar which engages a stud on the draw-tube. Provision for inserting a "1st order red" plate below analyzer. Plane of vibration of analyzer indicated by lateral crosshair.

10–Objectives. The higher powers to be parfocal, and all to be centered on the revolving nosepiece.

11–Eyepieces. Crosshaired, with stud to engage notch in draw-tube. Crosshairs must indicate planes of vibration of "crossed" nicol prisms.

12–Condenser. Abbe, with iris diaphragm, or separable condenser above polarizer.

The above essential requirements are met by the latest models of chemical microscopes by several makers, the chief differences being in the manner in which these features have been incorporated in the instruments.

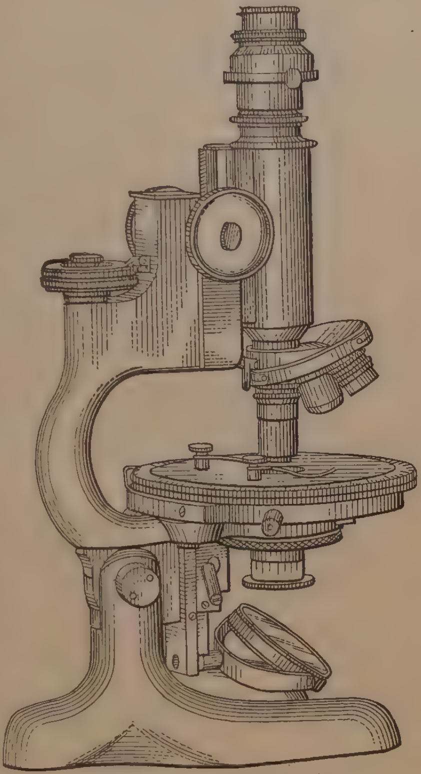

Fig. 19. Chemical Microscope (Bausch & Lomb).

The Bausch & Lomb Chemical Microscope (Fig. 19) possesses a standard substage ring, in which the polarizer or other illuminating apparatus can be readily mounted. A "1st order red" plate may be inserted between the eyepiece and the cap analyzer. A small mechanical stage may be attached to the rotating stage, so as to move with it. As a substitute for the ordinary polarizer, a nicol prism with a small separable condenser attached may be obtained, for use in illuminating with convergent polarized light in the study of interference figures. The upper lens of this condenser may be swung aside, to furnish illumination for fields larger than that of a 16-mm. objective. An iris diaphragm is attached below the polarizer.

A body-tube having the analyzer arranged to slide in or out, and with a slot for the insertion of a quartz wedge or other compensators, may be ordered instead of the standard tube. This equipment, together with the polarizer and swing-out condenser described above, makes the instrument equivalent to a simple type of petrographic microscope, and greatly extends its usefulness and convenience for the study of the optical properties of crystals.

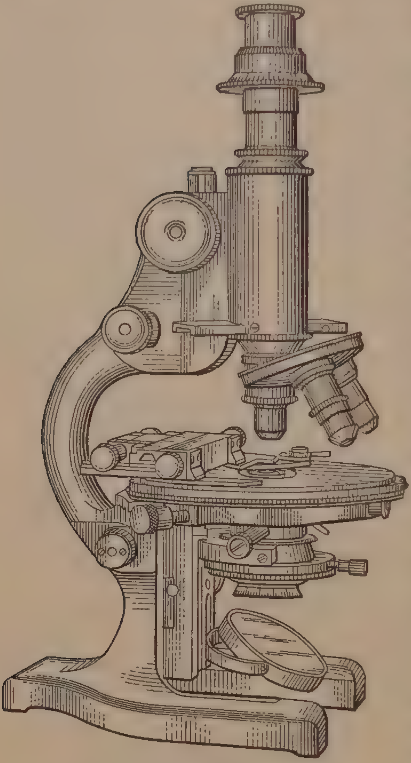

FIG. 20. Chemical Microscope (Spencer Lens Co.).

The Spencer Lens Company's Chemical Microscope (Fig. 20) is equipped with a fork-type substage, for rapid and precise interchange of the polarizer and other illuminating apparatus. Simple adapters, of standard diameter, permit any make of condenser or dark field illuminator to be used with the instrument. A slot, with dust-proof shutter, for compensators is provided in the lower end of the body-tube. The plane of vibration of the analyzer is indicated on its mounting — a great convenience in determining refractive indices of doubly refractive materials.

Instead of the simple polarizer, a polarizing apparatus may be obtained, which is particularly convenient for general work as well as for the study of the optical properties of crystals (Fig. 21). It carries a separable condenser, the top lens of which is easily swung aside in case it is necessary to illuminate a very large field. The polarizer may be readily removed, the condenser and iris diaphragm being left in place for illumination with unpolarized light; or the entire condenser may be removed from the polarizer. The illuminating apparatus is centerable by two screws, as an aid in obtaining symmetrically convergent illumination and in the examination of interference figures.

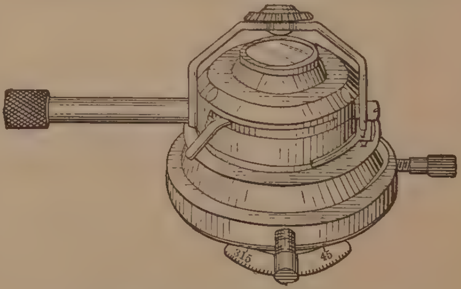

FIG. 21. Polarizer with Swing-out Condenser, for Convergent Polarized Light (Spencer Lens Co.).

The revolutions of the fine adjustment are indicated by a micrometer scale and the value of the divisions is marked in microns. The mechanical stage described on page 71 may be attached to the stage of the microscope so as to rotate with it.

The Leitz Chemical Microscope (Fig. 22) is equipped with a clutch-type centerable objective changer, C, but a revolving nosepiece can be substituted if desired. A small mechanical stage may be attached to the rotating stage. The slot for compensators is located between the eyepiece and the cap analyzer. A small condenser may be screwed on the top of the polarizer mounting, for use in observations with convergent polarized light.

The crystallographic microscope shown in Fig. 23 has been designed to afford the maximum facilities for general work, and particularly for the observation of optical properties with polarized light.[6] It incorporates the standard features of a "petrographic" microscope, with modifications to render it more suitable for the various investigations of the chemical laboratory.

The analyzer A is carried in a slide in the body-tube, and is rotatable through 90° to render it parallel with respect to the polarizer. It is fitted with a compensating lens to correct for shift of focus and astigmatism. The Bertrand lens B is provided with an iris diaphragm, for obtaining interference figures from only a part of the field, and can be focused by moving the draw-tube. The eyepiece is equipped with a movable eye lens, for focusing its crosshairs, and is keyed to the draw-tube.

Two centering screws C with locking device, at the lower end of the body-tube serve for adjusting the centration of the objectives. Compensators, such as the "1st order red" plate R and the quartz wedge Q, are inserted in a slot O which may be closed by a dust-proof shutter. A revolving nosepiece facilitates rapid interchange of the objectives, which are parfocalized and centered with respect to each other.[7] The fine adjustment is graduated.

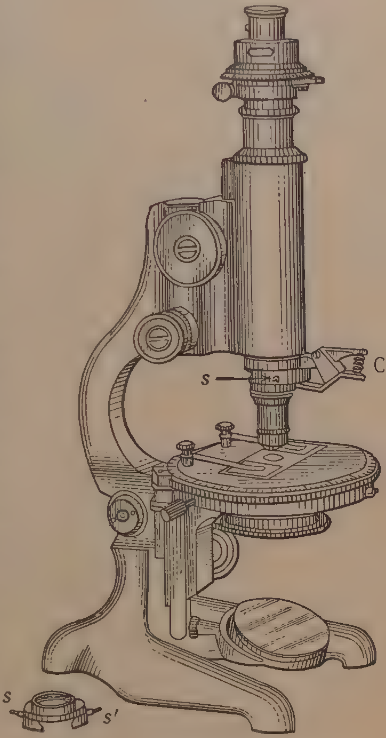

FIG. 22. Chemical Microscope (Leitz).

S, S, Screws for centering objectives of clutch objective changer, c.

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[6] This instrument may be obtained on special order from the Bausch & Lomb Optical Co., Rochester, N. Y.

[7] A convenient set of objectives, possessing exceptionally long working distances and large fields, is as follows: Leitz 24 mm., 0.21 N.A., 16.5 mm. working distance; Leitz 13 mm., 0.40 N.A., 3.4 mm. working distance; Watson $\frac{1}{8}$ inch, 0.80 N.A., 1 mm. working distance.

The stage S is particularly convenient for studies of small crystals; its entire top (95 mm. in diameter) is movable over a range of nearly 1 cm. by means of the two coördinate micrometer screws M. In examination at low powers the specimen can be moved about freely by hand, and when some detail is to be studied at a higher magnification, its centration is easily effected by the screw movements. The stage is graduated in degrees, and is provided with a vernier reading to 0.1°; its central aperture is 32 mm. in diameter, with a reducing disk having an opening of 19 mm. for use with small object slides.

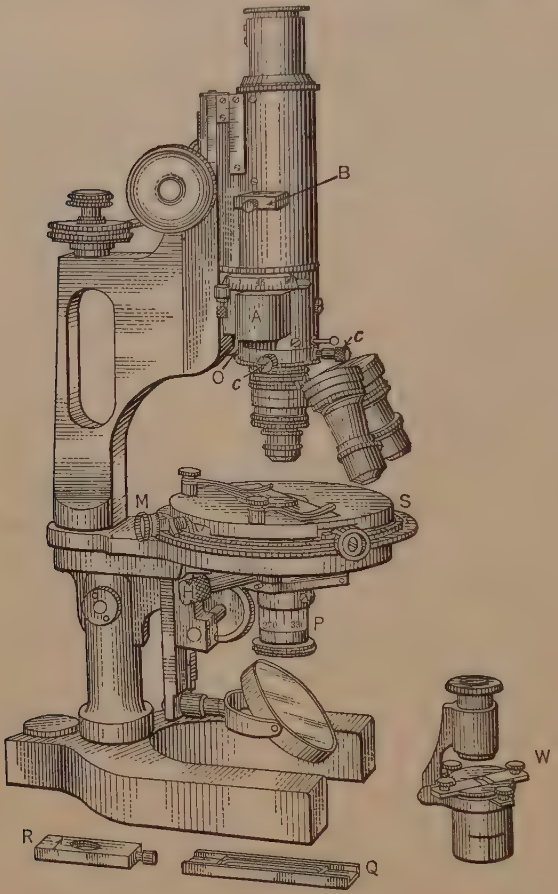

FIG. 23. Crystallographic Microscope (Bausch & Lomb) with Compensators and Wright Diaphragm for the Study of Interference Figures of Small Crystals.

The substage is of the rack and pinion type, and carries a nicol prism in a graduated revolving mount with a click at zero. The polarizer P is large enough not to restrict the aperture of the three-lens condenser mounted above it, and may be swung aside without disturbing the condenser. The condenser has a numerical aperture of about 1.10, and its focal length is great enough to allow illumination of the entire field of a 16-mm. objective. It possesses an iris diaphragm, and its two upper lenses may be turned aside by the knurled head H, to give less convergent illumination over a larger field.

The entire substage may be racked off and replaced by another with a ring of standard diameter, equipped with centering screws, to permit the attachment of a full size condenser, or a dark field illuminator or cardioid ultramicroscope.

BINOCULAR MICROSCOPES

Any microscope which enables both eyes to be used for observation of the same field may be called a binocular microscope, but the various types differ greatly in their advantages and in their optical construction. Ordinary binocular vision with the naked eye is superior to monocular vision, not only because the function is shared by two eyes but because the perception of distances is greatly facilitated. The appreciation of "depth" or "relief" in objects is due chiefly to stereoscopic vision, by which the images in the observer's eyes differ slightly.[8] All binocular microscopes have some provision for adjustment for **interpupillary distance**, which varies with different observers. This adjustment must always be carefully made, if the advantages of binocular vision are to be utilized. Some means of varying the focus or the magnification to compensate for differences between the right and the left eye is usually a part of the optical system. The eyepieces used should be selected or "paired" so as to be practically identical in their optical properties.

There is little reason to believe that proper use of a monocular microscope, especially if both eyes are alternately employed, causes any undue eyestrain; there is also little reason for believing that using both eyes simultaneously halves the strain for each. The chief justification for the use of binocular microscopes is in the interpretation of the structure of three-dimensional objects, for which stereoscopic vision is invaluable.

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[8] Siedentopf: *Zeits. wiss. Mikros.* **41**, 16 (1924).

Herbst: *Ibid.* **42**, 270 (1925).

Carpenter: *The Microscope and Its Revelations* (London, 1901), pp. 90–106.

Marshall and Griffith: *Introduction to the Theory and Use of the Microscope* (London, 1928), pp. 63–72.

Stereoscopic vision in the binocular microscope is dependent on the formation of slightly different images in each eye. This may be accomplished in two ways:

1–By means of a separate compound microscope for each eye, directed at the same object from slightly different angles.

2–By dividing and modifying the rays from a single objective, so that different images are produced in each eye.

Greenough binocular microscopes make use of the first of these methods, having two objectives, two body-tubes, and two eyepieces. The two systems converge upon the same field, and just as in vision with the naked eye their images are slightly different, resulting in true stereoscopic vision and a striking vividness of the third dimension.

Because the two compound microscopes cannot be directed at the same field if their objectives are of very short focal length and working distance, 18-mm. objectives are the highest powers commonly available. Their apertures are low, and their magnification is more or less " empty," especially if high powered eyepieces are used. However, Greenough binocular microscopes are not generally used in the study of fine detail, and this enlargement of the image aids considerably in manipulations under the microscope. The low aperature of the objectives gives great depth of focus, which is particularly advantageous in the examination of thick or irregular objects, and permits exceptional flatness of field and long working distance.

Greenough type binocular microscopes are further characterized by erecting Porro prisms in each body-tube, which serve to reinvert the image and to permit objects and movement to be seen " right side up." The adjustment for interpupillary distance is made by rotating the body-tubes with their offset eyepieces so that their eyepoints are centered with the pupils of the two eyes of the observer.[9]

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[9] The instruments recently manufactured by the Spencer Lens Company are given an exceptionally wide field by the use of wide body-tubes and large-diameter eyepieces. The convergence of the eyepieces is also made less than that of the objectives to minimize strain on the converging muscles of the eyes. The eyepieces of a recent model of Greenough binocular microscope made by E. Leitz, Inc. are parallel, for a similar reason.

High power condensers are superfluous for Greenough binocular microscopes, on account of the low aperture of the objectives used. However, a large plano-convex lens aids in securing uniform illumination, but the mirror (plane or concave) is ordinarily sufficient.

The stereoscopic, erect image, ample working distance, and great penetrating power of the Greenough binocular microscope render it ideal for all examinations where realistic appearance and ease of manipulation of the object are essential, and where high magnifications are not required. It forms an exceedingly valuable adjunct to a higher power " chemical microscope," and serves admirably to bridge the gap which too often exists between macroscopic and microscopic appearances.

Greenough binocular microscopes are especially useful in obtaining samples, separating ingredients of mixtures, preparing specimens for detailed study at higher magnifications, and in performing various mechanical operations under microscopic observation.

One of the most satisfactory types of this instrument is illustrated in Figs. 24, 25. The numerous possible positions of the microscope and arrangements of the stage and the object are evident without detailed discussion. Specimens of almost any conceivable shape may be examined; the stand, having an inclination joint, may also be used in a horizontal position for viewing the interior of chemical apparatus, furnaces, etc.

The prism chambers *c.c′* (Fig. 24) turn through a small arc in order that the eyepieces may be adjusted to the particular pupillary distance of each individual worker. When properly adjusted, the observer, looking into the microscope with both eyes open, should see a single circular field. If two overlapping fields appear, or if the field is blurred so that both eyes cannot simultaneously see it, the distance between the eyepieces should be readjusted. A shutter, which automatically remains open, is fitted just above the objectives. By means of the lever *s*, either half of the instrument may be closed, so that the user can ascertain if both eyes are actually in use. The shutter also serves in adjusting the focus. One of each pair of the higher power objectives is provided with a milled collar *m*. The left microscope is focused, the right being closed by the shutter. Then the shutter is reversed, and the focus further corrected by means of the collar *m* of the objective on the right side of the microscope. The instrument should now be equally well focused for either eye, and the object being studied should stand out stereoscopically and the image be clear and distinct.

The mounting of the body-tubes can be rotated, as shown in Fig. 25, in order that the worker may look into the instrument from the sides or front as the exigencies of the work may demand.

The magnifications obtainable with this type of microscope lie between about 3 and 330 diameters, with free working distances ranging from 70 mm. with the lowest power to 25 mm. with the highest. This is amply sufficient to permit working with a variety of tools upon objects lying on the stage. A revolving nosepiece carrying three pairs of objectives, any of which are readily interchanged, is one of the most recent conveniences added to stands of this type.

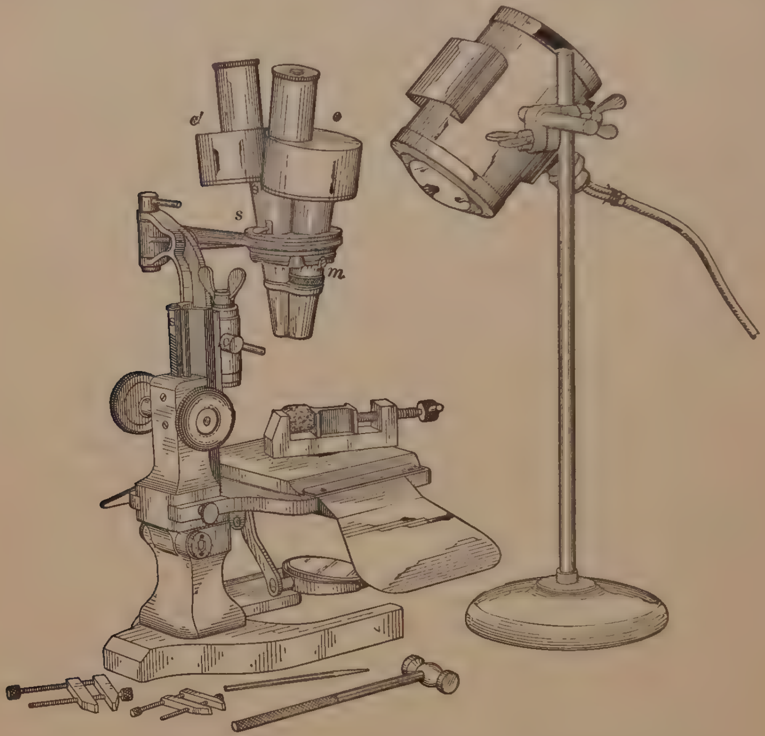

Fig. 24. Greenough Binocular Microscope (Spencer Lens Co.) with Lamp arranged for Inclined Illumination.

The stage is completely removable, and carries either a glass or a bakelite plate. The opening in the latter may be closed by a metal disk, giving a continuous flat surface. Beneath this opening is a rotating disk provided with one unobstructed opening for transmitted illumination, a ground-glass screen, an opaque white disk, and a black disk, any of which may be turned so as to give a variety of backgrounds against which to view the specimen. Removable hand rests attached to the stage greatly facilitate delicate manipulations.

A number of other types of stands, designed for the study of large or irregular objects (Fig. 25, E), are obtainable. A single optical system may be readily mounted on any of these, for use in the plant or laboratory.

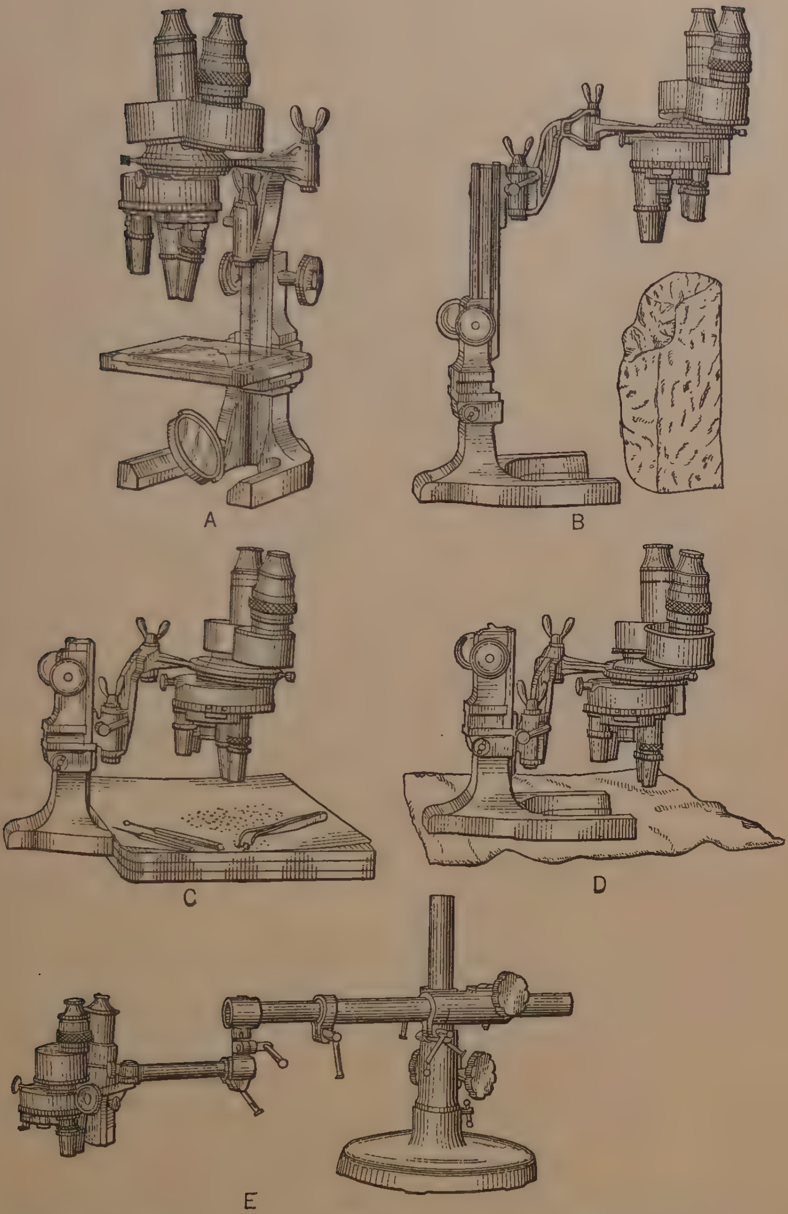

Fig. 25. Greenough Binocular Microscope with Wide-field Eyepieces and Rotating Objective Changer (Spencer Lens Co.).

Wide field attachments for Greenough binocular microscopes or special low power stereoscopic binocular magnifiers are particularly useful for the examination of large fields at magnifications of 10 diameters or less.

Monobjective binocular microscopes utilize the rays from a single objective, dividing them between two eyepieces. The division formerly was made at the back aperture of the objective, half of which served for each eyepiece. This involved a loss of resolution, for neither eye received the rays from the full cone of the objective. It is now customary to effect the division of light so that the full aperture of the objective is available for one eye, and usually for both. A semi-transparent reflector, covering the entire back aperture of the objective, sends a full cone of rays, of approximately half intensity, to one eyepiece, and lets the remainder pass through to the other (Fig. 26 A). No loss of resolving power results from such an arrangement, and each eyepiece receives the same image.

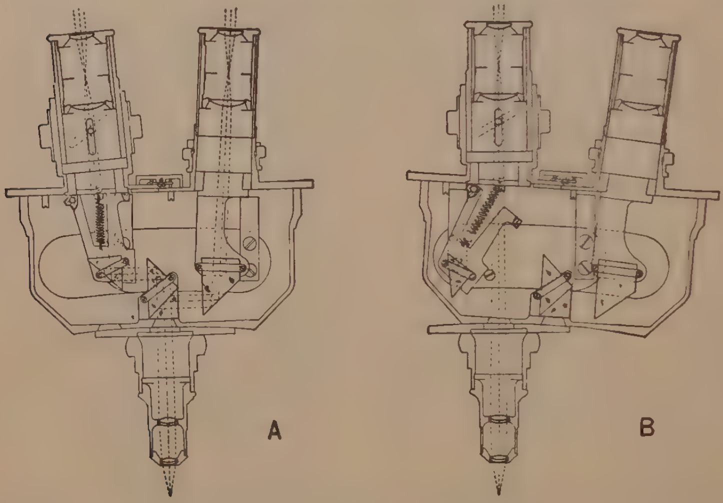

Fig. 26. Combination Body-tube of Monobjective Binocular Microscope (Spencer Lens Co.).

A — as a binocular. B — as a monocular.

Various methods are used to furnish different images to the two eyes, in order that stereoscopic vision may be obtained. It is possible, by adjusting the distance between the eyepoints to somewhat *less* than is required, to render them excentric with the pupils of the respective eyes. The irises act as aperture diaphragms at the eyepoints, to give the effect of unilateral oblique illumination from opposite directions, in each eye. The two retinal images are therefore shaded differently, and the impression of stereoscopic vision is received by the observer.

The excentric position of the eyepoints and pupils of the eyes, essential in the above procedure, may be eliminated by the use of diaphragms which are mounted at the eyepoints and cover the inner half of the aperture. The effect of unilateral oblique illumination in each eyepiece, and of stereoscopic vision, may be enhanced by the use of a diaphragm with two excentric openings, in the aperture of the condenser. This diaphragm gives oblique illumination from opposite directions and reinforces the effect of the eyepoint aperture diaphragms so that a very striking three-dimensional image is obtained, even at high powers.

Another method of obtaining stereoscopic vision at high magnification, when the eyepoint is small and diaphragms cannot be accurately adjusted to it, depends on the introduction of a suitable diaphragm in the body-tube. An image of the objective aperture is formed in the plane of this diaphragm by an auxiliary lens.[10] This lens also serves to erect the image.

All monobjective binocular microscopes may be used with standard objectives and eyepieces of any power, with a condenser, and with dark field or other special illumination. High resolving power may be obtained, especially if no supplementary aperture diaphragms are employed. Their optical tube lengths are generally somewhat longer than those of monocular microscopes, on account of the reflecting prisms which lengthen the path of the light. Interpupillary distance adjustment is provided by means of a rack-and-pinion to move the eyepieces laterally (involving a change of tube length and magnification) or by an excentric mounting which permits their being rotated to different distances. The length of one body-tube is further adjustable, to compensate for differences in the two eyes of the observer.

For highly critical work, micrometry, or photomicrography, the monocular microscope is preferable to the monobjective binocular, and most manufacturers provide a means of replacing the binocular tube and prisms by an ordinary monocular body-tube, without altering the position of the objective.[11]

Binocular attachments are also available, by means of which the ordinary microscope may be converted into a binocular. These devices fit in place of the regular eyepiece, and are capable of yielding very satisfactory stereoscopic vision, but they add considerably to the height of the microscope and are therefore inconvenient to use.

Binocular vs. Monocular Microscopes. — The Greenough binocular microscope has a field of usefulness which is unique, on account of its erect image and true stereoscopic effect. The virtues of the monobjective binocular microscopes are less marked. If they are not used so as to give stereoscopic vision, they possess only the doubtful advantage of dividing the image between both eyes. If aperture diaphragms are used to give a stereoscopic effect, resolution suffers somewhat, but the realistic appearance of objects in three dimensions aids greatly in the interpretation of their structures.

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[10] Lihotzky binocular stereo attachment, made by E. Leitz, Inc.

[11] The Spencer Lens Company manufactures a most convenient binocular, which by a simple lateral displacement of the body, becomes a monocular with all the light going directly to one eyepiece (Fig. 26, B).

The proper adjustment of a binocular microscope, involving interpupillary distance and independent focusing for each eye, is somewhat inconvenient and time-consuming, but the peculiar advantages of the instrument cannot otherwise be realized. The use of binocular microscopes requires that *both* eyes be maintained exactly centered with respect to the two eyepoints. This necessitates holding the head and neck very rigid and causes greater muscular fatigue than does the use of a monocular instrument.

COMPARISON MICROSCOPES

It happens not infrequently that it is found necessary to compare carefully several preparations of different samples. This is especially true in quantitative microscopy and in the estimation of particle-size. With ordinary microscopes it is necessary to place first one specimen and then another under the microscope, making drawings, measurements, and mental notes of the appearance of each in turn. Comparison of the observations is largely a matter of visual memory, the process is not easy or rapid, and the results are not always trustworthy even when obtained by an expert. Photomicrography offers a fair solution, but here again the time required and the additional manipulations involved prevent its general application.

Comparison microscopes are so constructed that the images of two different objects are brought into juxtaposition, so that they are simultaneously visible to the observer.

Comparison eyepieces (page 37) afford an inexpensive means of obtaining the advantages of a comparison microscope, provided two compound microscopes with suitable stands and optical equipment are available. Accurate comparison work requires that both microscopes should have optical systems of identical character. The stands should be of similar height, and should be capable of being placed sufficiently close together.[12]

Objects to be compared carefully by means of this instrument must necessarily lie in the same plane, in order to be seen at identical magnifications. If the difference in thickness is marked, the specimens may be supported on auxiliary stages (Fig. 53) adjusted to bring their upper surfaces to the same level. This, however, is possible only when no substage condenser need be employed.

Comparison microscopes are in some respects superior to comparison eyepieces, since their two body-tubes are permanently united on a single stand, and attached to an eyepiece in which half the field of each is seen. The optical system is essentially the same as in the use of the comparison eyepiece with two separate microscopes.

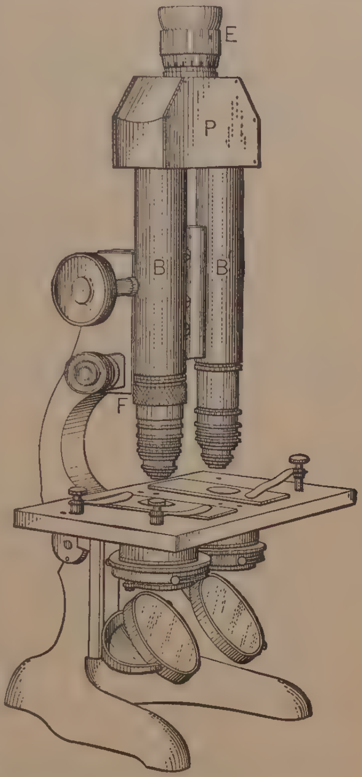

Fig. 27. Comparison Microscope (Leitz).

The comparison microscope of Leitz (Fig. 27) consists of two body-tubes, B,B′, with objectives, and an eyepiece in which half of each field is brought to juxtaposition by a system of reflecting prisms P. The fine line of division between the two halves of the fields is vertical, and may be focused sharply by means of the rotating collar of the eyepiece E. Both coarse and fine adjustments are provided for focusing the two microscopes simultaneously; a screw collar, F, permits focusing one objective independently of the other, in case the object slides are of different thickness.

The stand possesses a single stage, with two openings, and two complete substages, each equipped with condenser, iris diaphragm, and mirror. The illumination can therefore be adjusted to give equal illumination in the two halves of the field, or regulated to permit the size, shape, and color of each preparation to be exhibited under optimum conditions.

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[12] Comparison eyepieces are seldom made sufficiently large to be superimposed on stands with extra large rotating stages.

Simple magnifiers are often useful for manipulations, separating materials, or for low power examinations in the field. They give erect virtual images magnified as high as 120×, though the lower powers are more convenient to use on account of their longer working distance and greater depth of focus. Simple magnifiers must be placed very close to the object (less than their focal length); hence adequate illumination is difficult with the higher powers. The eye must be placed as near to the lens as possible, in order to see the whole field.

Simple magnifiers are designated by their focal lengths, and by their magnifications on the basis of 250-mm. image distance. The better grades are corrected for chromatic and spherical aberrations. The mountings may be suitable for pocket use, or for attachment to a focusing support (Fig. 28). Magnifiers for measuring carry an engraved scale in the lower focal plane.

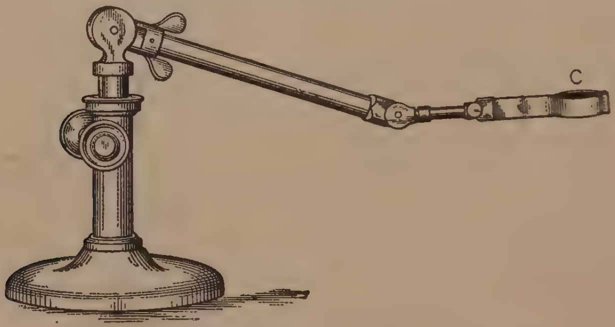

Fig. 28. Focusing Holder for Simple Magnifiers.

Monocular erecting microscopes (sometimes called dissecting microscopes) are optically similar to one-half of a Greenough binocular microscope. An erect image may be obtained with an objective of any power, and manipulations under the microscope are facilitated by the fact that the image is not reversed.

The **wide field attachment** made by the Bausch & Lomb Optical Company consists of a special high-eyepoint eyepiece and an objective mounted together, the whole constituting a low power (6×–10×) compound microscope with exceptionally wide field. The attachment is inserted in the upper end of the draw-tube of the microscope, the ordinary objective and eyepiece having been removed. It may be obtained with a micrometer scale in the eyepiece, for use as a measuring microscope in general laboratory practices.

Mechanical Stages. — In order to facilitate moving objects with precision and to insure certainty in covering a given area in quantitative or search work, some form of device permitting accurate coördinate movement in the plane of the stage is essential. Mechanical stages are designed for these purposes, and are almost indispensable accessories in a great variety of microscopic work.

Attachable mechanical stages are preferable for general chemical work, especially since, if liquids are spilled, they may be completely removed from the microscope for cleaning. If possible, they should be constructed so as to rotate with the stage, and when removed should leave its entire surface unobstructed. The mechanical stage should move freely, without rubbing against the surface of the stage. A piece of thick paper may be temporarily inserted beneath it, when it is being attached, to insure proper clearance. The range of motion should be at least sufficient to cover a 25-mm. square in the center of a 25 × 75-mm. object slide.

Both movements of the mechanical stage should always be accurately graduated and provided with verniers, for measurement of specimens (page 396) and for taking the coördinates of a particular point on a preparation in order to facilitate future reference to it. Systematic examination of the entire area of a preparation is frequently required, as in quantitative analyses of mixtures, determinations of particle-size, search for foreign particles or characteristic structures in foods, feeds, drugs, and other materials. To accomplish this, the specimen must be observed in the microscope while being displaced just sufficiently to expose an adjacent field to view. For "sampling" the preparation, fields may be chosen at intervals of, say, 3 mm. in successive rows 3 mm. apart.

A type of attachable mechanical stage which is particularly suitable for use with the chemical microscope is shown in Fig. 29. It is fastened by a pin and a simple lock-screw to the top of the rotating stage, and can be turned with the latter. Both of the coördinate movements are graduated, and of ample range; the transverse one depends upon a rapid-acting screw, and the other upon a rack-and-pinion. When the mechanical stage is not in use the surface of the microscope stage is flat and unobstructed.

The mechanical stage shown in Fig. 30 is also very convenient, though not so easily attached or cleaned as the one just described. In this type, a narrow slot is cut into the microscope stage. The opening thus made is provided with beveled grooves at the sides, into which slips a sliding metal plate, M, provided with coördinate movements. When a perfectly plain stage is wanted,

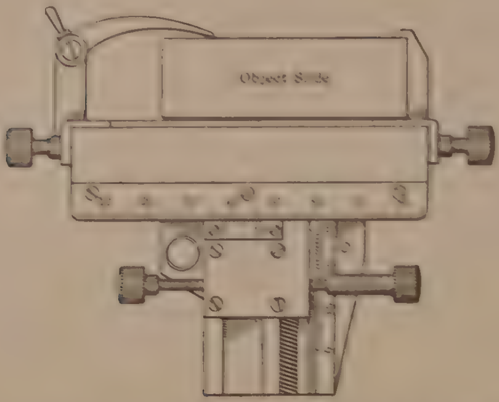

FIG. 29. Mechanical Stage. Attachable to Rotating Stage (Spencer Lens Co.).

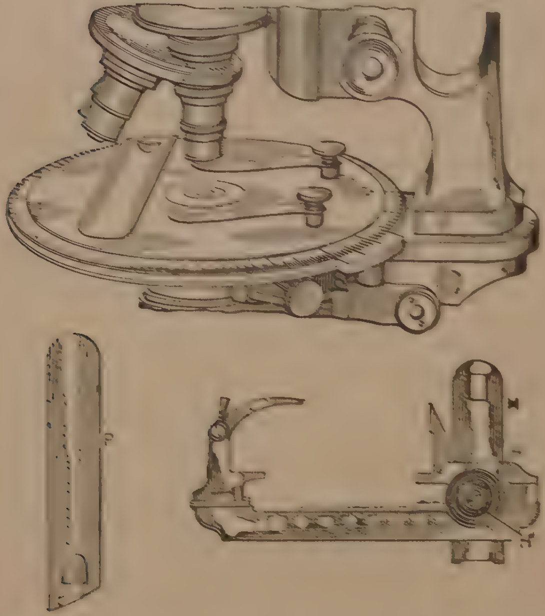

FIG. 30. Mechanical Stage. Attachable to Rotating Stage (Spencer Lens Co.).

the mechanical stage is removed, and a plain black metal plate, P, is inserted in its place. The coordinate movements of the stage are made by rack-and-pinion actuated through the coaxial milled heads H H'. This stage may be seen in place in Fig. 56.

The rotating stage with movable top, described on page 66, is the least obtrusive and most convenient of all mechanical stages for centering small particles or for movements over a limited area.

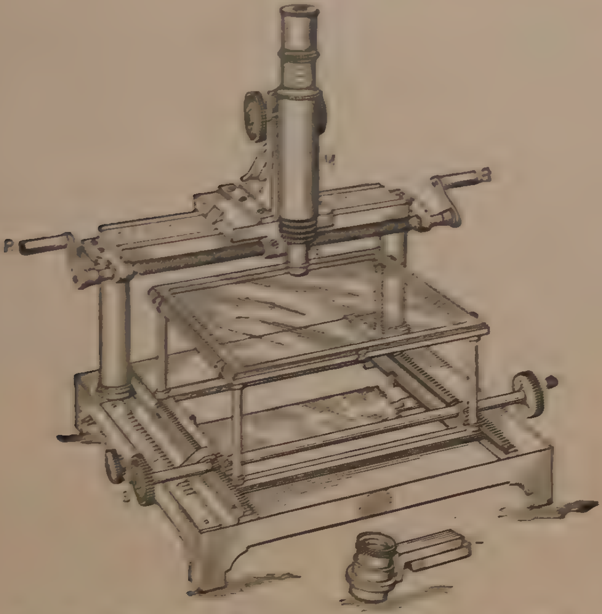

Fig. 31. Traversing Microscope, for the Examination of Large Areas (Leitz).

The "traversing microscope"[13] shown in Fig. 31 is useful for systematically examing large samples of powdered materials, surfaces, etc. Its construction is obvious from the illustration. The microscope is made to traverse the entire stage by means of the screws R and S. A low power simple magnifier L is first employed, and when a particle is found which requires further study the compound microscope M is mounted in place of the lens L. Since the fields are concentric, this transition is easily effected many times in examining the area.

The compound microscope is provided with a rack-and-pinion coarse adjustment, and with a rotary-screw movement F which serves as a fine adjustment.

[13] Made by E. Leitz, Inc.

CHAPTER III

ILLUMINATION OF TRANSPARENT OBJECTS. LIGHT SOURCES

Illumination. — The microscope does its part if it images accurately and clearly the appearance of the object on which it is focused. This appearance, however, is not an invariant property of the object but depends upon external factors as well as upon the inherent characteristics of the object itself. The most important of these external factors is the illumination. It governs resolution, the visibility of fine details and of the object as a whole, the "naturalness" of the microscopic image, and the ability to interpret this image in terms of the structure from which it originates. Proper illumination technique is indispensable in critical microscopy, whereas unintelligent use of the wrong methods of illumination may neutralize the worth of the best objectives and eyepieces and may give images that represent but a small portion of the detail which is observable or that may actually falsify the apparent character of the object.

The study of the optics of illumination is an essential part of microscopy, particularly for the purposes of the chemist, since the variety of materials which he examines is so great that the whole range of illumination procedures must be at his command. Routine methods of illumination are adequate for routine examinations where they have been tested, but inventiveness and ingenuity are required in dealing with objects presenting any unusual features.

The microscopist should constantly strive to acquire experience and develop judgment in the recognition of structures by examining each of his specimens under varying kinds of illumination and noting how its aspect is affected. By so doing he will increase many-fold his ability to obtain the maximum information from each microscopic image and also his confidence in the validity of his observations on novel and unstudied objects, such as continually come to the chemist, for which no rules of procedure have ever been laid down.

The precise description of appearances, so important in all chemical investigations, is possible only if the conditions of their observation are given with equal accuracy, and if the effects of varying types of illumination are understood. Exact and unequivocal terminology is particularly essential in recording microscopic observations and the illumination by which they were made.

Visibility of Objects. — In most cases, the pattern of light and shade and color which is seen as a microscopic image exists in reality *at the object,* and could be observed with the naked eye, if the structure were large enough. The microscope serves chiefly to magnify this "stage picture."[1] The development of the shadow and color pattern which serves to reveal the object and to differentiate it from the surrounding material is governed by:

1–The structure of the object.
2–The optical properties of the object.
3–The character of the illumination.

The **structure of the object** obviously governs the image by determining its shape or outline. Furthermore, the structural details of the object properly appear in the image as patterns of light and shade or of color which may be traced back to the structure itself. The dimensions of these details determine their resolution and their visibility. Usually the relationship of the structure to the image is of the sort familiar to us in ordinary unaided vision and presents few new difficulties of interpretation. Certain anomalies of appearance are discussed in the following pages, and in Chapter VI.

The **optical properties of the object** are of the utmost importance in image formation, and must be considered in relation to its structure and to the illumination supplied. Experience with ordinary objects, as seen by the unaided eye, does not go far in the interpretation of microscopic images, since other optical properties are utilized. The optical environment (such as the mounting medium) may vary considerably, and the methods of illumination are usually more complex.

Microscopic objects are seen by virtue of:

1–Refraction images.
2–Color images.
3–Polarization images.
4–Reflection images.
5–Diffraction patterns.
6–Fluorescence.
7–Combinations of the above.

The **refractive index** of a transparent object determines its refraction image, which consists of a shadow pattern representing its various surfaces and structures. If the refractive index of the surrounding medium is identical with that of the object, no refraction image is possible, and the object will be invisible unless other properties (such as color, polarization, etc.) can be utilized to reveal its form.[2]

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[1] The many factors which govern the formation of the "stage picture" are discussed in detail by A. E. Wright: *Principles of Microscopy* (London, 1906), pp. 3–39.

A considerable degree of transparency may be gained by the choice of a mounting medium of the proper refractive index, so that the interior structure of the object may be revealed. If, on the other hand, the difference between the refractive indices of the object and the surrounding medium is too great, the shadow outline is unduly emphasized and the specimen may appear almost opaque in places, so that its inner structure is hidden.[3]

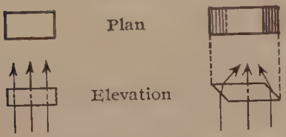

Fig. 32. Origin of Shading in Images of Transparent Objects.

The **refraction images** of colorless transparent objects consist of light and dark areas which may be traced back to their bounding surfaces. Wherever the light has not been directly transmitted, but has been deviated by refraction or reflection at some minute surface, this surface appears dark in the image and helps to reveal the structure of the object (Fig. 32). If the structural details are well within the limit of resolution, their integrated action on light may be thought of as simple refraction or reflection, but where their dimensions are very small (approaching the wavelength of light) their action must be analyzed in terms of diffraction. Diffraction by transparent substances is wholly dependent on the relative refractive indices of object and environment.

The form and structure of the object are not difficult to interpret in simple cases, for the microscopist can always utilize the principles of refraction, reflection, and diffraction to work out the structure of unfamiliar objects. If the surfaces are not too complex, and if the direction of illumination is known, the origin of each bit of light and shade in the image may be traced to some detail of structure in the object (page 176). To the experienced worker this process may become almost unconscious.

Transparent areas in the image indicate surfaces which are more or less parallel in the object, and which lie perpendicular, or nearly so, to the axis of the microscope. If the inclination is great, these surfaces may appear less transparent, or even dark if the refractive index of the substance is less than that of the surrounding medium. Single inclined surfaces and surfaces meeting at an angle appear more or less opaque. Plane surfaces seen edgewise appear as dark lines. Curved surfaces have shading of varying intensity.[4] Microscopic observations of colorless crystals, unstained textile and paper fibers, emulsions, precipitates, white pigments, and other finely divided materials depend ordinarily on refraction images, which are interpreted as indicated above and in Chapter VI.

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[2] Ice immersed in water is nearly invisible for this reason.

[3] The considerations which govern the selection of a mounting medium are discussed on p. 167.

In general, the action of the individual surfaces is obscured if they are illuminated from many directions simultaneously. Their shadows are lighted up, and their outlines become transparent and present little contrast with their surroundings. In thoroughly diffuse light, from all directions at once, a transparent colorless object is completely invisible.[5] Even a wide-angled illuminating cone from a high-aperture condenser may cause a serious loss of visibility.[6] High aperture objectives tend to give images of lessened contrast, for analogous reasons.

The color of a transparent material depends on its absorption of light of certain wavelengths and the transmission of the remainder.[7] The hue observed with transmitted light is properly called the **transmission** or **body color** (page 191). Since absorption is an internal property, the refractive index of the surrounding medium will not affect the color of a substance, and a colored object is visible microscopically even if mounted in a medium of identical refractive index. Under these conditions, the object is seen solely by virtue of its **color image** and exhibits a high degree of transparency at its boundaries.

Color images are little affected by the direction of the illumination, and suffer little loss of contrast when a wide-angle condenser is used, provided the light is not too brilliant. Colorless objects are frequently dyed or stained in order to obtain color images of them which are less dependent on the illumination and may reveal certain differences in chemical nature. The realism of color images and the ease of interpreting structures by them are usually distinctly less than in the case of refraction images, because of their similarity to colored silhouettes.

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[4] Spangenberg: *Zeits. wiss. Mikros.* **38**, 1 (1921) gives an exhaustive discussion of the optical origins of the various appearances at the boundaries of objects, under different conditions of illumination.

[5] Wood: *Physical Optics* (1919) p. 99.

[6] One might consider the central rays from the condenser as yielding a dark image on a bright field, and the highly oblique rays as furnishing dark field illumination (p. 43) which gives a bright image on a dark field. The combination of the two results in a very imperfectly contrasted refraction image.

[7] Colors depending on structure are discussed on pp. 192 to 196.

Color images, or combinations of color and refraction images, are the basis of microscopic observations of materials such as colored crystals, dyed or stained fibers, foods, leather, and other naturally or artificially colored substances.

The **double refraction** of optically anisotropic substances enables them to be seen by virtue of their polarization colors (page 280). Such polarization images do not depend upon the refractive index of the substance as compared with the surrounding medium, or upon its body color. The object is examined between crossed nicol prisms, and appears luminous and colored against a dark field.

Polarization images are restricted to doubly refractive objects, but are very useful since they utilize an additional set of properties, independent of the optical environment of the specimen. The visibility is high, inhomogeneities and differences of thickness or orientation are revealed, and adjacent isotropic material is rendered invisible.

Examination between crossed nicols, purely as a method of revealing structural features, is invaluable in the study of textile and paper fibers and other animal or vegetable tissues, plastics, or colloidal aggregates. The more precise applications of the phenomena of double refraction are discussed in Chapter IX.

The **reflecting power** of opaque objects renders possible the examination of their surface characteristics. If the hue of the reflected light is different from that of the incident light the substance exhibits a **surface color**, due to its **selective reflection** (page 191). The reflecting power of transparent substances is low, and is dependent upon their refractive indices; they are therefore usually examined by transmitted light. Metals have high reflecting powers, not appreciably affected by the refractive index of the surrounding medium; they are studied almost entirely by reflected light. The **reflection image**, obtained by appropriate illumination of an opaque object, may be interpreted by the application of the laws of reflection and diffraction. Its details correspond to differences in the reflecting power of various parts of the object (analogous to a color image, though not necessarily colored) and to the variously arranged reflecting surfaces of the specimen (analogous to a refraction image). The illumination of opaque objects by reflected light is discussed in Chapter IV.

Diffraction patterns (page 13) are important in the imaging of fine structures of either transparent or opaque objects, though negligible so far as the larger features are concerned. In ultramicroscopy (page 212) they are the sole basis of the image. The visibility of the diffraction pattern yielded by an object point depends upon its relative refractive index (if the object is transparent) and upon its reflecting power (if the object is opaque). The brilliancy of diffraction effects is enhanced by illumination of high intensity, and is made more striking by the absence of all extraneous light. This principle is applied in dark field and ultramicroscopic illumination (page 213).

Diffraction patterns and halos surrounding fine details of structure often give rise to anomalous effects which are likely to be very misleading. In order to avoid the falsification of minute dimensions, or the rendition of dark lines and points as bright, and vice versa, the utmost care must be taken in adjusting the focus as accurately as possible (Fig. 80).

Fluorescence or luminescence is the property, exhibited by certain substances, of emitting light of a different color from that used to illuminate them. Fluorescence is most marked with ultraviolet illumination, and may yield light of various colors that are more or less characteristic of the objects under examination and therefore useful for purposes of identification. The fluorescent light may be masked if transmitted illumination is used, hence dark field illumination (page 87) is commonly employed. The specimen appears self-luminous, not only at its surfaces but also in its interior, under these conditions.

A great many substances have been studied by means of the fluorescence microscope (page 95). Natural and artificial organic materials frequently show pronounced and characteristic fluorescence.

Most microscopic images utilize **combinations of the above properties** of objects. Indeed, special procedures may have to be employed in order to isolate the effect of one property of the object from those of its other properties. Certain types of images are mutually exclusive in practice, or interfere seriously with each other.

Pure refraction images can be obtained only from colorless transparent objects, or colored objects illuminated by light of their own color. The greater the difference in the refractive indices of object and surrounding medium, the greater will be the opacity of the shadows and the bolder the outlines of the object.

Pure color images require the elimination of all refraction effects by use of a mounting medium of the same refractive index as that of the object. If shadow outlines are present (particularly if they are broad and opaque) color observations may be difficult, since the hue of the object is visible only where light is able to pass through it. Color images are affected markedly by the hue of the illumination.

Polarization images are interfered with by refraction effects, which may render the object almost opaque, and by absorption, which may superpose the body color of the object upon its polarization color. Objects mounted so as to be as transparent as possible give the best polarization images.

Reflection images are very susceptible to changes in the direction of the illumination. They may also be color images, if the substance possesses the property of selective reflection.

Methods of Illumination. — The various methods of illumination which are used in microscopy are not peculiar to certain types of objects but are of general application. However, the procedure and the apparatus (though not the principles) of illumination are generally different for transparent and for opaque objects. For this reason the discussion of methods will be thus divided, but the reader is urged to keep in mind the numerous useful analogies in theory and technique which emphasize the essential unity of illumination principles.

The relationships between the different types of illumination are shown in Fig. 33, which also indicates the gradation and lines of demarcation between them.

ILLUMINATION OF TRANSPARENT OBJECTS

Objects which are appreciably transparent, at least in thin layers, or which can be made transparent by decolorizing, are usually examined by **transmitted light.** By its use the interior features are revealed in a manner which would not be possible if the object were studied by light reflected from its outer surfaces. The low reflecting power of most transparent substances is another reason against the use of reflected illumination.

Illumination by transmitted light is obtained from a light source or the image of a light source, placed on the side of the object which is away from the objective. The object is seen as a transparency, either as a color or a refraction image (or both), against an illuminated background. This is sometimes spoken of as **" bright field " illumination.**

Illumination by the Mirror. — In the ordinary microscope the light is reflected upward through the object by means of a reversible mirror placed below the stage. If the **plane mirror** is used, only the direction of the light is changed. Diffuse light from the sky remains diffuse, and comes to the object from all directions subtended by the area of the mirror. The intensity of the illumination is governed by the brightness of the light source.

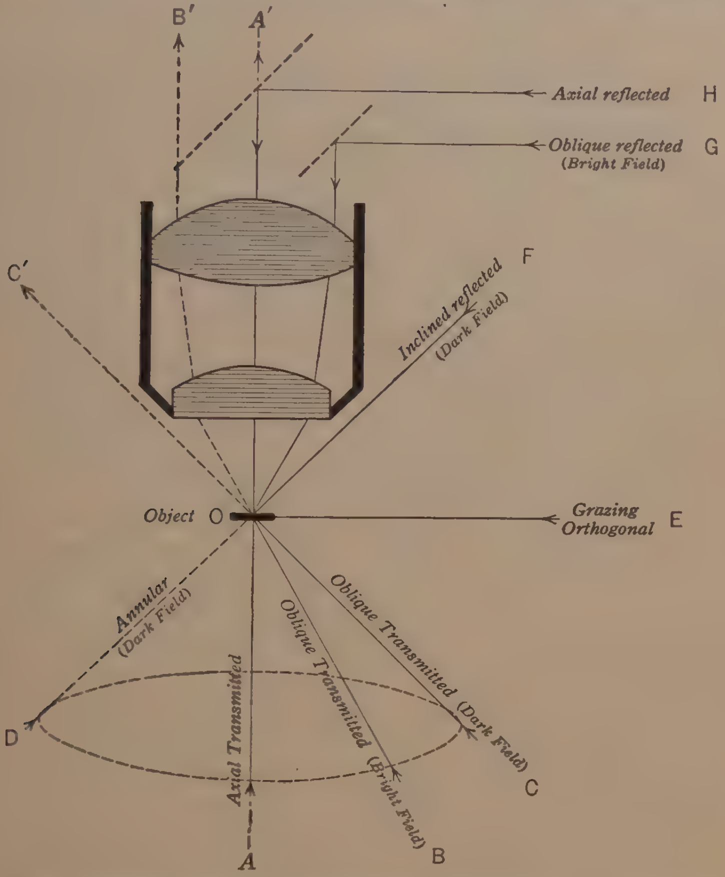
B′
A′
Axial reflected
H
Oblique reflected (Bright Field)
G
C′
F
Inclined reflected (Dark Field)
Object O
Grazing Orthogonal
E
Annular (Dark Field)
Axial Transmitted
Oblique Transmitted (Bright Field)
Oblique Transmitted (Dark Field)
D
C
A
B

FIG. 33. Diagram Showing Relationships of Various Types of Illumination.

The field illuminated is rather larger than the mirror unless a very narrow beam of light is supplied. If an auxiliary condenser is used to render the light parallel or convergent before it is reflected by the mirror, the illumination is much less diffuse and the field more sharply limited. The method of illumination by means of the plane mirror alone is suitable only for low power microscopy. Ground glass or white paper placed below the object will furnish similar diffuse illumination, over areas larger than the mirror.

The **concave mirror** may be used for illumination by transmitted light. It serves chiefly to converge the light upon the object, giving brighter illumination over a smaller field. The light is somewhat diffuse, under ordinary conditions.

If the mirror is centered with respect to the axis of the microscope, and tilted toward the light source so as to illuminate the field uniformly, the illumination is substantially **symmetrical** in that no more light comes upward in one direction than in another. This may be spoken of as **axial illumination**, but it must be borne in mind that the light may not be wholly parallel to the axis of the microscope but may strike the object at a variety of angles. The shading of the refraction image is uniform on all sides.

To test for the symmetrical character of the illumination a preparation of a viscous liquid (glycerine, gum arabic solution, oil, etc.) into which air has been beaten is focused sharply. A tiny spherical air bubble, in the center of the field, should show a bright center and a dark border which are perfectly concentric.[8] There should be no swaying of the image if the microscope is focused up or down.

If the mirror is swung to one side of the axis of the microscope, and adjusted so as to illuminate the field uniformly, **oblique illumination** is obtained. Here again the light is not strictly unidirectional though the intensity is much higher on one side of the object than on the other. This results in unsymmetrical shading and a realistic appearance of " relief " which is very useful in bringing out the third dimension of objects (Fig. 133). The use of oblique illumination in the determination of refractive index is given in Chapter XI.

Illumination by means of the mirror alone is suitable only for low power microscopy, where large fields must be studied and where fine details are not important. Because of their simplicity, the above methods are largely employed below 100×, and even at higher powers if polarized light is used. Greenough binocular microscopes require only this diffuse illumination, obtained either by the mirror, by a translucent screen of ground glass, or by a white background beneath the object.

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[8] Gage: *The Microscope* (1925), p. 112.

Illumination by means of the Condenser. — The use of the mirror alone does not permit accurate control of the direction and intensity of the illumination, or of the area of the field illuminated. Only a small degree of convergence of light is possible (< 0.25 N.A.), and objectives of high aperture cannot be "filled with light." For work at medium or high powers, the use of some form of **substage condenser** and diaphragm is essential. The optical properties of condensers are discussed in Chapter I.

Axial transmitted light (A, A', Fig. 33) may be readily obtained by means of the condenser. By adjusting the mirror to give symmetrical illumination, and closing the iris diaphragm to a small aperture, only rays which are practically parallel to the axis of the microscope are transmitted. Lowering the condenser has an effect similar to closing the iris diaphragm (Fig. 14, A, f). The direction of the light reflected into the aperture of the condenser influences the direction of illumination, and it is always advisable to test for strictly axial light by means of an air bubble (page 82) or by noting whether there is swaying of the image as the focus of the microscope is changed. If the condenser is not centered with respect to the axis of the microscope it may be impossible to obtain strictly axial illumination.

If the iris diaphragm is not attached to the condenser but is in a separate excenterable mounting, it is important that the position where it is exactly centered should be denoted by a "click" in the mechanism, and that it should remain centered if the de-centering apparatus is rotated. The centration of the diaphragm may be tested by observing it at the back aperture of the objective, the condenser being absent.

By properly adjusted axial illumination, the direction of the light is definitely controlled. The shaded outlines of refraction images are symmetrical, and much bolder than with diffuse or convergent light. Contrast is increased, but diffraction patterns are accentuated, and resolution is lessened.[9]

Strictly axial illumination is valuable in the examination of objects of low visibility, which give faintly outlined refraction images. It is also useful in the interpretation of shading in terms of structure, in the observation of polarization colors, and in testing refractive indices (page 369).

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[9] It should be borne in mind that, although light enters the object only parallel to the axis of the microscope, surfaces and other fine structures may deviate it, so that it leaves the object at various angles. If only axial rays are to be dealt with (as in the accurate observation of polarization colors, p. 283) an objective of very low aperture should be used.

Convergent transmitted light (*A*, Fig. 14) is obtained when the condenser is placed so that the object lies in or near its upper focal plane (page 41). The condenser and mirror should be properly centered, in order that the illuminating cone may be symmetrical with respect to the axis of the microscope. The degree of convergence is regulated by the iris diaphragm, or by lowering the condenser. These manipulations simultaneously affect the brilliancy of the illumination, as well as its convergence. The diaphragm is ordinarily adjusted by trial, but for most preparations it need not be closed to less than two-thirds the aperture of the objective, as observed by looking down the draw-tube.[10] The size of the field illuminated depends on the focal length of the condenser and on the size and distance of the source of light (page 41).

The mirror should always be placed so as to furnish symmetrical illumination. Swinging it to one side will give oblique illumination, the direction of which is not easily controlled on account of internal reflections in the condenser. If necessary, the air bubble or the swaying of the image may be used as tests for proper adjustment of the mirror.

If the condenser is off center, symmetrically convergent light may not be obtainable. With poorly corrected condensers, a considerable quantity of stray light may come to the object, and decrease the clearness of the image. Closing the diaphragm to give greater contrast may cause a loss of detail, as explained on page 13.

Condensers of long focal length (page 41) do not require as perfect centration as those of higher aperture, on account of the large fields which they illuminate. The size of field illuminated by a short-focus condenser may be increased considerably by lowering the condenser so that the image of the light source is enlarged and diffused by being far out of focus. The effective aperture of the condenser is decreased by this procedure. Long-focus condensers are very frequently focused considerably above the plane of the object, to increase the area illuminated (*B*, Fig 14).

If the light source is not of uniform intensity or flatness (incandescent lamp filament, crater of arc lamp, sky with clouds or trees) its image, projected in the field of the microscope, will furnish uneven illumination. This may be remedied by throwing the condenser just sufficiently out of focus to diffuse the image uniformly.

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[10] The relative angular cone of the condenser, as diaphragmed or lowered, may be observed at the back aperture of the objective (p. 47).

The use of auxiliary condensers and diaphragms, to permit the regulation of the size of field and intensity of illumination without affecting its degree of convergence, is discussed on page 98.

Symmetrical convergent illumination is employed in practically all critical work at magnifications greater than 100×, and is highly advantageous at lower magnifications. It is essential in the examination of interference figures of crystals (page 288). The increased intensity of light is useful in the study of relatively opaque materials such as colored minerals and crystals, dyed fibers, thick sections of leather or other tissues, and similar specimens. The exact control of intensity and of degree of convergence is a great aid in the study of the internal structures of transparent substances, such as textile and paper fibers, food materials, crystals, and other microscopic objects. Careful regulation of the diaphragm opening will reveal numerous features which would otherwise be overlooked. This adjustment is fully as important as focusing the microscope, and each specimen should be studied with varying illuminating cones to be sure that the best possible visibility and definition have been obtained.[11]

Oblique transmitted light (BO, CO, Fig. 33) should always be used as a supplement to symmetrical or axial illumination, the appearance of the object being noted as the conditions are varied. Oblique transmitted light is quickly and easily obtained with the ordinary condenser by screening all but one edge of its aperture (page 43). If no condenser is used, the mirror may be swung off center to give oblique illumination.[12] Neither of these methods gives a very sharply defined illuminating beam, and the light is not strictly unidirectional.

Unilateral oblique transmitted light is best obtained by means of an excenterable iris diaphragm below the condenser. A unidirectional illuminating beam of any degree of obliquity in any azimuth may be adjusted with considerable exactness, and maintained fixed for photographic purposes.[13] Highly oblique (dark field) illumination (COC′, Fig. 33) may be obtained, if the condenser is of distinctly greater aperture than the objective (page 43). The unsymmetrical shading which aids so greatly in achieving three-dimensional appearances with oblique illumination is enhanced if objectives of low numerical aperture are used, since less of the light refracted by the object can be included.

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[11] The rôle of the condenser in "critical illumination" as an aid to resolution is discussed on pp. 12 and 44.

[12] The optics and methods of oblique illumination are discussed by F. E. Wright: *Amer. Jour Science* (4), **35**, 63 (1913).

Oblique illumination should always be obtained by passing from symmetrical convergent or axial illumination, the mirror being kept centered if the condenser is used. Any of the above methods are suitable for most work, and since the change in the shading can be observed as the transition is made to oblique illumination they are invaluable as aids in the interpretation of refraction images. Emulsions, crystals, fibers, starches, and any objects possessing superposed structures may be very realistically observed and photographed, so that their three-dimensional character is apparent. In general, the direction of obliquity should be such as to send light *crosswise* of the structures (fissures, striations, crystal faces) which are to be accentuated. By rotating the stage, the changes in shading may be clearly brought out and the best orientation selected.[14]

Annular oblique illumination (page 43) is obtained by means of a hollow cone of rays, oblique in all azimuths and converging upon the object. In the case of examinations by transmitted light, the obliquity is such that no direct rays are included within the angular cone of the objective, and **"dark field" illumination** results (CO, DO, Fig. 33). The cone of rays must be focused on the object; it must be symmetrical; and it must be centered. If a condenser with central stop and annular opening is used, it must be well corrected, and of high aperture, in order to permit the use of objectives of considerable resolving power. For low powers the ordinary Abbe condenser, with central stop, is satisfactory but for higher powers special **dark field illuminators** are greatly to be preferred. The use of the condenser for differential color illumination is described on page 94.

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[13] A special three-leaved rotatable shutter made by the Spencer Lens Company is designed to permit the regulation of oblique illumination.

[14] The use of oblique illumination in the determination of refractive indices is discussed on p. 369.

Images with Dark Field Illumination. — The image obtained with dark field illumination is apparently self-luminous against a dark ground. It is essentially a *negative* of the refraction image with bright field illumination. Every surface, fissure, or particle in the object which deflects light appears bright instead of dark; even faint outlines appear brilliantly contrasted against the black background. The brightness of the image depends on the intensity of the light source, the darkness of the field, the concentration of light by the illuminator, the light-grasping power of the objective, and the reflecting, refracting, and diffracting power of the object, as governed by its index of refraction as compared with that of the surrounding medium. Colored objects, if mounted so as to give refraction images, show their body color faintly, by virtue of internal reflections which cause some of the light to be transmitted by them. Objects showing surface color (selective reflection) or fluorescence may exhibit different colors by dark field illumination than by transmitted light.

The aberrations of objectives are particularly apparent with dark field illumination, and false color effects may be observed if residual chromatic aberration is not taken into account. The resolving power of a given objective with dark field illumination may be fully as great as that with bright field illumination,[15] provided the precautions indicated in the following pages are carefully observed. Objectives of the highest numerical aperture cannot be used, but the enhanced contrast in the image may compensate for this limitation.

Types of Dark Field Illuminators. — Dark field illumination necessitates an annular illuminating cone of considerably higher aperture, and a dark cone of slightly higher aperture, than that of the objective. Ordinarily the convergence is obtained by reflection, since chromatic aberration is thereby eliminated. For this reason dark field illuminators are sometimes called **" reflecting condensers."**[16] The necessary reflection is effected by two principal types of construction:

1–A single paraboloid surface.
2–Two spherical surfaces.[17]

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[15] Berek: *Zeits. wiss. Mikros.* **41,** 1 (1924).
Siedentopf: *Ibid.* **32,** 1 (1915).

[16] The history of reflecting condensers is given by Siedentopf: *Zeits. wiss. Mikros.* **24,** 382 (1908).

[17] Siedentopf: *Kolloid Zeitschrift* **37,** 327 (1925).

Paraboloid illuminators (Figs. 34, 37), such as those manufactured by Bausch & Lomb and by Zeiss, consist of a block of glass shaped in the form of a segment of a paraboloid of revolution. Parallel rays are reflected from its curved surface (by total reflection) and converge at the focus of the paraboloid. The central rays are stopped, so a hollow cone of light results. The obliquity of the illumination may be increased by reducing the annular aperture by means of an iris diaphragm, *d*, so that only rays near the focus are reflected.

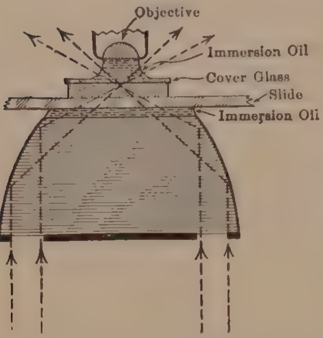

Fig. 34. Dark Field Illumination, with Homogeneous Immersion Objective.

Paraboloid surfaces cannot be ground with absolute accuracy, so the convergence of the light is not perfect. This results in an appreciable thickness at the apex of the illuminating cone, where the object is placed, so that the field of a 16-mm. objective may be almost entirely illuminated. The intensity of illumination suffers somewhat, because such a large area is illuminated. The lack of perfection of convergence gives a certain latitude in focusing the illuminator.

Two spherical reflecting surfaces (Fig. 35) are used in a number of types of " bispheric " dark field illuminators.[18] The rays enter through an annular aperture, are reflected (by total reflection) from a spherical cavity to a second (silvered) spherical surface, and thence to the focus of the illuminator.[19]

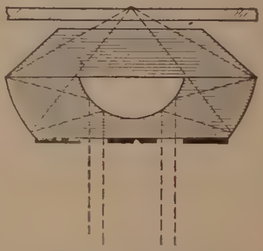

Fig. 35. " Bispheric " Dark Field Illuminator (Leitz).

The spherical surfaces may be ground with great accuracy and such illuminators possess a minimum of aberrations, and a very sharply defined focus.[20] The convergence of light is so nearly perfect that a relatively small field is illuminated, and the object must be placed exactly at the apex of the cone of rays. The intensity of illumination varies with different makes of instruments.[21]

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[18] Such as those of Spencer Lens Company and Leitz.

[19] The principle of these illuminators was developed by von Ignatowsky: *Zeits. wiss. Mikros.* **26**, 387 (1909).

[20] Their corrections are discussed by Smiles and Barnard: *Jour. Roy. Micros. Soc.* **269,** 365 (1924).

Manipulation of Dark Field Illuminators. — Dark field illuminators require somewhat greater care in adjustment than do ordinary condensers, but the necessary experience is easily acquired if principles are understood. As a preliminary to work with the dark field illuminator the reader is urged to familiarize himself with the discussion by Gage[22] and if possible to examine the illuminating cone by means of a block of uranium glass.

In all dark field illuminators the obtuseness of the illuminating cone is such that all rays strike the upper surface of the illuminator at an angle which is greater than the critical angle of glass in contact with air. Consequently, just as in the case of high-aperture condensers (page 45) these oblique rays will not emerge from the illuminator into air. It is therefore necessary to use a **contact liquid** between the top surface of the illuminator and the under surface of the slide. The object should not be mounted "dry," for the same reason. Homogeneous immersion oil is preferable as a contact liquid. The use of water limits the maximum numerical aperture of the illumination to 1.33, and may entail the loss of part of the cone. If an objective of numerical aperture greater than 1.3 is used, the object should be mounted in glycerine ($n = 1.47$), Canada balsam ($n = 1.54$), or other highly refractive medium, in order that the light may not be totally reflected at the upper surface of the slide before it reaches the object.

The **numerical aperture of the objective** determines whether dark field or bright field illumination will be obtained from any given illuminator. Theoretically, the numerical aperture of the objective need be only very slightly less than that of the dark cone of the illuminator. Practically, this is not strictly true, on account of the finite area of the field which must be illuminated,[23] and the stray light from internal reflections and diffraction at the edges of the reflecting surfaces. It is generally advisable to employ an objective the numerical aperture of which is at least 0.10 smaller than that of the dark cone of the illuminator, in order to obtain a satisfactory black field which is free from haze. Most manufacturers state the maximum numerical aperture permissible with their illuminators, and it is well to keep 10 to 20 per cent below this value.

Until recently, 0.9-N.A. objectives were about the maximum that could be used with dark field illuminators. There was little justification for the use of oil immersion objectives, since their aperture had to be reduced by means of inserted diaphragms (Fig. 36) with a loss in resolution which rendered their resolving power no better than that of dry objectives. At present a number of dark field illuminators are on the market which will function satisfactorily with objectives having numerical apertures of 1.10, 1.20 or even 1.30.[24] Special homogeneous immersion objectives of these apertures may be obtained, but it is preferable to use an objective which is fitted with an iris diaphragm, so that its aperture may be decreased just enough to give a satisfactory dark field with the minimum sacrifice of resolving power (Fig. 5). By opening the diaphragm, the full aperture of the objective is instantly available for bright field examinations.

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[21] The cardioid condensers of Zeiss and Bausch & Lomb (p. 222) and the Jentsch ultracondenser of Leitz (p. 225), which are used as ultramicroscopes, are essentially the same in principle as the above dark field illuminators.

[22] *The Microscope* (Dark Field Edition, 1925), Chapter XII.

[23] Berek: *Zeits. wiss. Mikros.* **40**, 225 (1923).

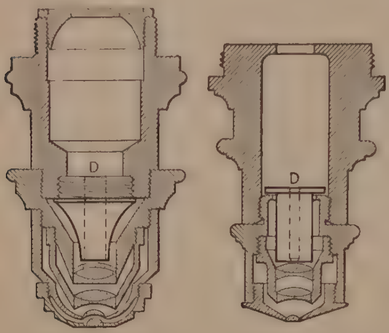

FIG. 36. Methods of Reducing Numerical Aperture of Objectives for Dark Field Studies.

D, D — removable diaphragms.

The **centering** of dark field illuminators is exceedingly important, particularly in the case of those having spherical reflecting surfaces. The tiny area illuminated must coincide exactly with the field of the objective, which is of course very small in the case of high powers. Centering screws (*c*, Fig. 37) are provided in the mounting for this purpose. Some illuminators have the location of their field indicated by a small circle engraved upon the upper surface. Before placing the object in position, this circle (which is invisible when covered with a contact liquid) must be centered with the field of the objective to be used, by means of the two centering screws. A lower power objective may be used for preliminary centering; unless the microscope is provided with a well centered revolving nosepiece,

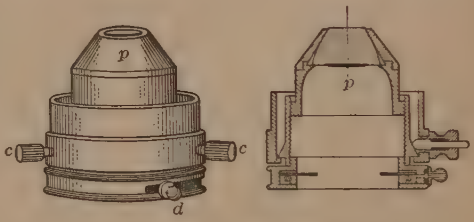

FIG. 37. Paraboloid Dark Field Illuminator.

[24] The "Bicentric" condensers of Leitz permit the use of objectives of 1.15 N.A. The "Luminous Spot-ring Condenser" of Zeiss may be used with objectives of 1.30 N.A. The preparation must be mounted in a liquid of $n > 1.45$. This rules out aqueous suspensions but non-aqueous mounting liquids may be used satisfactorily for many substances such as pigments and fillers; colloidal material in oils or reinforcing pigments in rubber may be examined at high resolution by this instrument. The "Cassegrain" dark field condenser (Watson & Sons) and the "Special Focusing Dark Ground Illuminator" (of R. & J. Beck) also permit the use of high-aperture objectives. The "Bicentric" condenser of Leitz is obtainable in quartz, permitting the use of ultraviolet light for fluorescence or extreme resolution.

the higher power objective should be substituted in the same opening. A centering adapter or sliding objective changers are useful aids in such work. Occasionally the substage ring of the microscope is so far out of center that the range of the centering screws of the illuminator is insufficient. Replacing the illuminator after rotating it 180° may remedy this.

If the illuminator is not provided with an engraved circle to indicate its field, centering must be carried out with a preparation in place. The mirror is adjusted to give symmetrical illumination, the illuminator is focused so as to give as small an area of light as possible, and this area is rendered concentric with the field by means of the two centering screws.

Lack of centration manifests itself not only in a non-uniformly illuminated field but also in a highly unsymmetrical appearance of the bright portions of the image. Diffraction patterns of fine structures become so distorted as to prevent any satisfactory focusing or adequate interpretation.

The focusing of a dark field illuminator is even more important than the centration, for if the rays converge above or below the object it lies in darkness and is invisible. The height of the apex of the cone of rays above the top surface varies with different types of illuminators. Obviously, the slide used must not be thicker than this " working distance " or the object cannot be brought close enough to receive light. Most manufacturers specify the maximum slide thickness which is permissible with their instruments. In general, the higher-aperture illuminators require rather thin slides (1 mm. or less). If too thin a slide is used, a thicker film of immersion oil (or a cover-glass or two, oiled together) will serve to maintain the proper optically homogeneous system. Certain dark field illuminators are adjustable to give variable working distances (Fig. 38); adjustment for thick slides ordinarily involves a decrease in the maximum permissible aperture of the objective. The range of movement of the substage ring must be sufficient to permit raising the top of the illuminator level with the upper surface of the stage. If necessary, the optical system of the illuminator may be raised in its mounting to render this possible.[25]

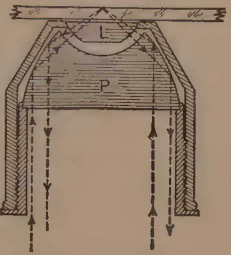

FIG. 38. Dark Field Illuminator, Adjustable for Slide-thickness (R. & J. Beck).

The focusing of the microscope must be carried out with more care than is necessary in bright field work, since the numerous diffraction patterns broaden out rapidly as the point of sharpest focus is passed. Spurious images and misinterpretations of structure may result if the focus is not adjusted to give maximum sharpness. The effective depth of focus of the objective is apparently less than with bright field illumination, on account of the accentuation of all out-of-focus effects. Thin preparations are preferable to thick ones, for similar reasons.

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[25] A substage fine adjustment, as provided on the larger stands by Spencer Lens Company, is a great convenience for the accurate focusing necessary in dark field microscopy.

Light sources for dark field illumination should be of high intensity, particularly if faint outlines or submicroscopic particles are to be revealed. A beam of approximately parallel rays, of sufficient diameter to fill the aperture of the illuminator, may be obtained from any concentrated light source by means of an auxiliary condenser (page 97). For visual work at low powers, a concentrated filament incandescent lamp (not frosted) may be sufficient, but an arc light of 4 or 5 amperes is preferable. In ultramicroscopy more powerful arcs, or even the direct rays of the sun, from a clockwork heliostat, are necessary to reveal exceedingly fine particles.[26]

The mirror should be adjusted to furnish symmetrical illumination. This condition may be recognized by means of the annular trace of the hollow cone of light at the surface of the illuminator, or, if a preparation is in place, by the circular shape of the area illuminated. The concave mirror should never be used with dark field illuminators, nor should any attempt be made to compensate for poor centration by displacement of the mirror.

The influence of each of the different factors discussed in the foregoing pages may be rendered very vivid by means of a block of uranium glass, which shows the path of the rays with various positions of the mirror and emphasizes the need for proper centration and focusing. In order to expedite the adjustments it is well to have at hand a permanent preparation of some material which yields good results with dark field illumination, as for example diatomaceous earth. The light source, mirror, and illuminator may be mutually arranged to give the best possible appearance, and then the test slide may be replaced by the preparation to be studied. Such preliminary study is a great time-saver in the examination of specimens of unknown structure and helps to eliminate the danger of misinterpretation or failure to secure a satisfactory image.

Cleanliness is essential, particularly in critical work or in the examination of submicroscopic particles. Any specks or smears on slides or cover-glasses appear as diffraction patterns or fog and decrease the blackness of the field so that faint details are lost. Slides and cover-glasses must be exceedingly clean and free from dust. Lens paper should be handled as little as possible and applied with a minimum of pressure, in order that fatty material from the fingers may not be forced through its pores on to the glass.[27]

The **preparation** should be as thin as feasible in order that it may be almost entirely in focus. Any extraneous matter or objects out of focus will cut down the blackness and contrast of the field. The special cell[28] shown in Fig 39 enables a uniformly thin layer of a suspension to be examined while protected from evaporation or alteration by the air. The construction of this cell is essentially similar to that used with the cardioid ultramicroscope (Fig. 102), but the cover-glass is thin enough to be used with ordinary objectives.

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[26] A cooling cell or heating-absorbing glass should be used, to avoid heating the illuminator or the specimen (p. 108).

[27] Special methods of cleaning, for ultramicroscopic work, are described on p. 224.

[28] Manufactured by E. Leitz, Inc. Obtainable in glass or quartz.

Collodial suspensions or other finely divided material should not be too concentrated, or the out-of-focus diffraction patterns will give a hazy field and destroy contrast. Air bubbles in the preparation or in the immersion liquid of illuminator or objective are particularly harmful, for they reflect light so strongly as to nullify the dark field illumination in their vicinity, even if they do not appear in the field.

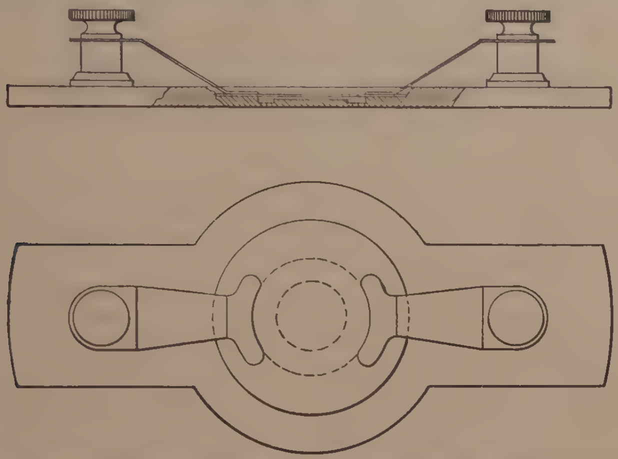

FIG. 39. Cell and Holder for Use with Dark Field Illuminators (Leitz).

Combination Dark and Bright Field Condensers. — It is often desirable to compare the appearances of details under dark and bright field illumination, as a check on the interpretation of the dark field image. A number of illuminators are on the market which permit transition to bright field illumination without disturbing the preparation.[29] A strictly critical transmitted illuminating cone of high aperture is not obtainable with these "change-over" condensers, but they are satisfactory for ordinary observations where a high-aperture condenser is not necessary.

Choice of Dark Field Illuminators for Chemical Work. — The dark field illuminator is a most useful accessory in general microscopic work such as chemists are called upon to do. It is particularly valuable where the contrast of the preparation is low, and in addition may serve as an ultramicroscope. For low power examinations the paraboloid or the "change-over" dark field illuminators are preferable. If very fine details must be resolved (such as zinc oxide or carbon black, or products of the "colloid mill") an illuminator which permits the use of high aperture immersion objectives should be selected. Both types may well be purchased, if much dark field work is done, since those of highest aperture cannot be used on aqueous preparations.

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[29] Those of Leitz and Zeiss follow the construction of their regular models, with removable central stops, and iris diaphragms for the control of the transmitted light.

By the use of a sufficiently powerful light source, such as an arc lamp with its crater imaged in the aperture of the illuminator by means of an auxiliary condenser, any good dark field illuminator can be made to function as an **ultramicroscope.** Particles far beyond the limit of resolution and much too fine to be visible by bright field illumination can be revealed if the illuminator is carefully adjusted, taking into account all the precautions indicated on the preceding pages. Many colloidal phenomena may be studied by such an arrangement, but the limit in the revelation of submicroscopic particles can be attained only by the use of ultramicroscopes, certain types of which are nothing more than specially corrected bispheric dark field illuminators (page 88).

Differential Color Illumination, as devised by A. E. Wright and by Rheinberg[30] is closely similar in principle to dark field illumination. The most common arrangement consists of a colored transparent disk and a colored transparent annular screen, both of which are inserted in the lower focal plane of a refracting condenser. The disk functions as a central stop, to control the color of the field; the annular screen, as an annular aperture, to control the color of the light deflected by the object into the microscope. For instance, if the disk is blue and the annular screen red, the object will appear red on a blue background. Complementary colors are commonly chosen for the illumination; the darker is better used for the central stop, in order to give greater contrast. The dimensions of the central color disk must be such that the cone of rays which it colors is wholly included by the objective. The annular screen must give a hollow cone of colored light none of which will come within the angular aperture of the objective. Various other arrangements of stops may be devised.[31]

Although differential color illumination is little more than a curiosity in the study of colorless objects, it appears to have considerable possibilities as a means of increasing contrasts in colored preparations. By a proper choice of colored screens, ingredients of one hue may be rendered almost invisible, while those of another may be boldly defined. The particular color combinations have to be decided by trial, in each instance.[32]

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[30] *Principles of Microscopy* (1906), p. 19; *Jour. Roy. Micros. Soc.* **1896**, 373. Spitta: *Microscopy* (1920), 175–178.

[31] Hausman: *Trans. Amer. Micros. Soc.* **41**, 51 (1922) uses reflected and transmitted light of different colors.

[32] Further discussion of the illumination of colored objects is given on p. 101.

Fluorescence Microscopy. — Outfits for the examination of microscopic material by its fluorescent light (page 79) are manufactured by a number of firms.[33] The illuminating apparatus (Fig. 40) is a refracting condenser of quartz, in each case. The light source R is either a mercury arc or else a powerful arc between iron electrodes or carbons impregnated with a mixture of nickel and other salts; it must be rich in ultraviolet radiation. The visible light is eliminated by solutions of copper sulphate and *p*-nitroso dimethylaniline, combined with a screen of blue *Uviol* glass. A screen of violet Ultra glass, U (made by the Corning Glass Works, Corning, N. Y.) is more convenient than the above arrangement. An auxiliary condenser of quartz Q serves to concentrate the radiation on the aperture of the condenser. A quartz prism P is used instead of the mirror of the microscope. The optical system must be transparent to ultraviolet radiations as far as the object, so quartz or *Uviol* glass slides must be used, with glycerine as the contact liquid between the condenser and the preparation.

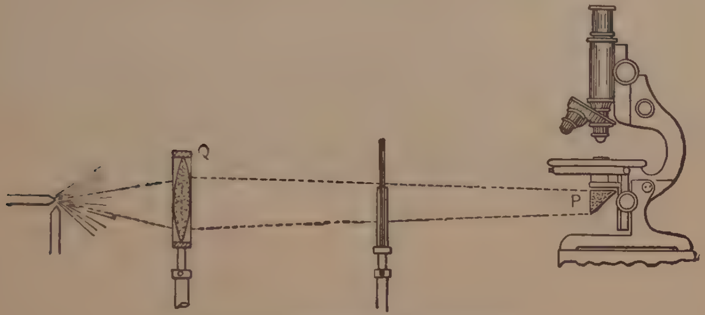

Fig. 40. Fluorescence Microscope.

Cover-glasses, objectives and eyepieces may be of the usual materials, since the object is viewed by visible light.

The illuminating system is the distinctive feature of a fluorescence microscope. Its various parts may be assembled and used with any microscope, since quartz condensers are purchaseable separately from various makers.[34]

Stray fluorescence, from immersion oil, glass lenses, or the retina of the eye will decrease the contrast of the object. The first cause is remedied by the use of glycerine as a contact liquid; the second and third, by dark field illumination. If bright field illumination is used for greater intensity, the fluorescence of the objective, eyepiece, and eye may be minimized by the use of cover-glasses of yellowish *Euphos* glass (Zeiss) which are practically opaque to ultraviolet light. A filter of Corning *Noviol* or similar glass above the eyepiece or goggles of the same material are important for protecting the eye from injury by ultraviolet radiations which are not completely absorbed by the lens system of the microscope.

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[33] Reichert, Bausch & Lomb, and Zeiss.

[34] Dark field illuminators of *Uviol* glass or of quartz are made by Leitz.

The adjustment of a fluorescence microscope is greatly facilitated by the use of a permanent preparation of a strongly fluorescent substance such as anthracene, "vaseline," barium platinocyanide, or a uranium salt. A block of uranium glass is also very useful as a means of revealing the path of the invisible ultraviolet rays.

Fluorescence microscopy has great potential usefulness as a means of differentiating substances which are apparently similar even under the microscope. Different kinds of cellulose, animal fibers, plant tissues, foodstuffs, powdered minerals, dyed materials, and organic or inorganic chemicals may show strikingly different fluorescence colors. Small amounts of impurities, in heterogeneous or homogeneous mixture, may be revealed, and differences in chemical treatment may be indicated.[35]

Combinations of Illumination Methods. — In a great deal of microscopic work, the appearance of the object may be rendered more realistic if more than one method of illumination is employed. Particularly in photomicrography it may be desirable to combine transmitted and reflected illumination. The "silhouette" appearance of opaque particles by bright field illumination may be relieved by "high lights" supplied by reflected light. Judicious balancing of the two methods of illumination is necessary, in order that each may contribute its share to the picture.

AUXILIARY CONDENSERS

The efficiency of any method of illumination is markedly influenced by the character of the beam supplied to the condenser of the microscope. All types of substage condensers are constructed to utilize practically parallel rays. Although it is feasible to depend on light directly from a light source, the solid cone of radiation subtended by the aperture of the condenser is very small, and the intensity of illumination may be too low for certain purposes, such as dark field illumination, photomicrography, or examination of strongly absorbing objects. By placing an auxiliary condensing lens of relatively large diameter in front of the light source, a wider cone of rays may be included by it and transmitted to the condenser of the microscope. The angular aperture, focal length, and location of the auxiliary condenser govern the beam supplied by it to the microscope; it should preferably be corrected for spherical aberration.

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[35] See Heimstädt: *Zeits. wiss. Mikros.* **28**, 330–7 (1911); Lehmann: *Ibid.* **30**, 417–70 (1913); Wasicky: *Pharm. Post.* **46**, 877 (1913); Lenz: *Zeits. anal. Chem.* **54**, 27 (1915); Barnard: *Nature* **106**, 378 (1920); Emich: *Mikrochemie* **3**, 27 (1925); Robl: *Zeits. angew. Chem.* **39**, 608 (1926); Klein and Strebinger: *Fortschritte der Mikrochemie* (1928), p. 189; Metzner: *Das Mikroskop* (1928), p. 281; Danckwortt: *Lumineszenz-Analyse* (Leipzig, 1928).

Approximately **parallel rays** will result if the light source is small and is located at the focal point of the auxiliary condenser (Fig. 41). These rays may be focused by the microscope condenser to give an image of the light source F, in the plane of the preparation F_1. Critical illumination (page 44) may be secured either by the substage condenser alone, or combined with an auxiliary condenser in this manner.

If the beam of light from the auxiliary condenser is larger than the diaphragm opening of the condenser of the microscope, it may be rendered slightly convergent by moving the auxiliary condenser away from the light source. However, the beam should not be concentrated to a smaller compass than that necessary to fill the aperture of the substage condenser, or unsymmetrically convergent illumination may be supplied

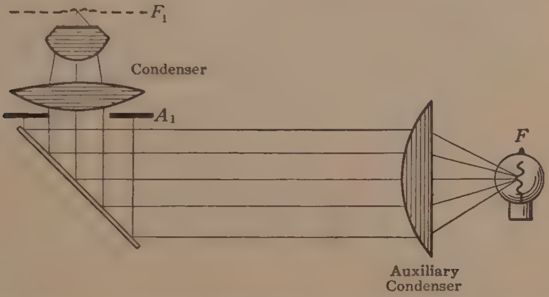

FIG. 41. Auxiliary Condenser, arranged to furnish Parallel Rays for Critical Illumination.

Auxiliary condensers mounted on suitable stands for the above method of illumination are frequently called "bull's eye" condensers. If uncorrected they are usually plano-convex and should be used with the plane side next to the light source, in order to minimize their spherical aberration. Many lamps sold for microscope illumination are equipped with such lenses in focusing mounts, so as to furnish either a parallel or a converging beam.

The iris diaphragm A_1 of the substage condenser acts as an **aperture diaphragm** and simultaneously varies the convergence and the intensity of illumination. No provision is made for regulating the area of the illuminated field other than by changing the distance or dimensions of the light sources imaged therein.[36] By means of a slightly different procedure, still further control of the illumination is possible.

Köhler's method of illumination[37] is characterized by:

1–The projection of an image of the light source, in the plane of the diaphragm of the substage condenser.

2–The projection of an image of a diaphragm in front of the light source, in the plane of the object.

An auxiliary condenser, with attached or separate iris diaphragm F, accomplishes this illumination as shown in Fig. 42. It should be of such focal length and so placed that the image of the light source is just large enough to furnish a uniform area the size of the aperture of the substage condenser A_1; this furnishes light of maximum intensity. The image must ordinarily be somewhat enlarged, if the light source is small and the diaphragm of the substage condenser fully opened. The diaphragm in front of the auxiliary condenser should be so placed that it is imaged by the substage condenser in the plane of the object F_1. This diaphragm functions as a **secondary light source**, of variable size; and by opening or closing it the area of the illuminated field is regulated. It is thus possible to illuminate no more of the object than that visible in the microscope, and to decrease "glare" very materially by closing this **field diaphragm.**

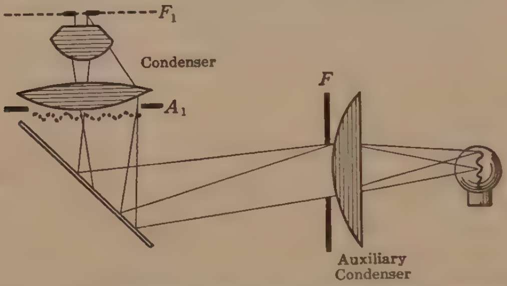

FIG. 42. Auxiliary Condenser and Field Diaphragm, arranged for Köhler's Method of Illumination.

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[36] Herbst: *Zeits. wiss. Mikros.* **42**, 290–301 (1925) discusses this inadequacy, and the use of auxiliary condensers in general.

[37] Köhler: *Zeits. wiss. Mikros.* **10**, 433 (1893).

The auxiliary condenser used in either of the above procedures should be of fairly short focal length and large aperture, but to function with maximum efficiency should be corrected for spherical aberration.[38] The auxiliary diaphragm may be mounted on a separate stand, but is usually placed in close proximity to the auxiliary condenser; the adjustment of the substage condenser serves to focus its image in the plane of the object. The diaphragm of the substage condenser functions, as usual, as an **aperture diaphragm**, and also serves to regulate the intensity of illumination.

Although there has been some discussion on purely theoretical grounds as to the relative merits of ordinary "critical illumination" with or without an auxiliary condenser, and Köhler's method of illumination, certainly there is no question as to the practical advantages of the latter.[39] The convenience of a (secondary) light source of easily variable size and the ability to adjust the area of the illuminated field are invaluable. Illuminating systems based on Köhler's method are widely employed for photomicrography, metallography, and microprojection, at medium or high powers. Its further application will be discussed in connection with the special apparatus used in each of these fields.

LIGHT AND LIGHT SOURCES FOR MICROSCOPY

The light source, as the initial element in the optical system of the microscope, merits consideration in connection with illumination methods. Its chief requisites are:

1–Adequate and controllable intensity.
2–Constituent radiations of the appropriate wavelengths.
3–Manipulative convenience.

Intensity is important in microscope illuminants on account of the large area over which the final image is spread. The increased numerical aperture and light-grasping power of objectives used for high magnifications help to compensate for this loss of intensity, but the apparent brilliancy of the image in the microscope is rather less than $\left(\frac{\text{N.A.}}{0.005 \times \text{magnification}}\right)^2$ times the object.[40] The condenser serves to concentrate the radiations from the light source upon the object but, even so, a high initial brilliancy is necessary. Light from a white sky is adequate for low and medium powers, but for high magnifications, particularly for photomicrography, microprojection, and ultramicroscopy, brighter sources are necessary. The approximate order of brightness of some of these is as follows: gas mantle (inverted), incandescent lamp, tungsten arc lamp, A-C. carbon arc, D-C. carbon arc, D-C. metal-coated carbon arc, direct sunlight (brightest).[41] The nature of the illuminating system markedly affects the intensity of light delivered to the object.

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[38] The "special collimator" of Leitz consists of several lenses; the focal length of the combination may be varied, to regulate the size of the image of the light source projected by it.

[39] Hartridge: *Jour. Queckett Microscopical Club* (2) **14**, 73 (1919) demonstrates that these methods are especially good.

The brightness suitable for visual microscopy will vary with the color and opacity of the object, and with its reflecting power if vertical illumination is used. According to F. E. Wright[42] the field should be no brighter than is necessary, and should be limited in area by field diaphragms at the light source and in the eyepiece. Provision should be made for moderating the intensity of illumination without affecting its aperture and convergence; this may be done by the introduction of smoked or neutral tint glass, color filters, or ground-glass screens, near the light source. Other methods of regulation, such as varying the electric current of an incandescent lamp, may affect the color of the radiation.

In photomicrography at high magnifications, especially in metallographic work, highly intense illumination is necessary in order to cut down the required exposure time. In microprojection, the brightness of the projected image must be sufficient to render it visible even in an imperfectly darkened room. Ultramicroscopy is limited chiefly by the available intensity of light.

Constancy and reproducibility of illumination are important in extended investigations, especially in photographic or ultramicroscopic studies. Daylight is particularly unsatisfactory in

[40] Beck: *The Microscope.* Part II (1924), p. 90.

[41] Comparative numerical brightnesses are given in Metzner: *Das Mikroskop* (1928), p. 140, but these are of limited application on account of the wide range of luminous efficiency of each type of lamp and the variation in relative brightness for different wavelengths.

[42] Resolving power and Contrast Sensitivity of the Eye. *Jour. Optical Soc. Amer.* **2**, 101–7 (1919).

this respect. Artificial light sources, if the current is constant, are far more useful.

The **constituent radiations** of a light source for microscopy should be different for various purposes. For visual work involving observations of color, white light of composition similar to daylight from a white sky is generally preferable. Daylight itself is commonly used, but it must be borne in mind that its hue varies considerably depending on the blueness of the sky. Artificial light is more or less yellowish as compared with daylight, and may interfere with accurate discriminations of yellows and blues; it is probably somewhat more tiring to the eyes than daylight. However, few microscopists care to be restricted to ideal daylight conditions, and many laboratories are so situated as to necessitate work by artificial light.

An excellent substitute for daylight is obtainable by the use of *Daylite* glass[43] in connection with an incandescent electric lamp. If the proper thickness of glass suitable to the intensity of the light source is chosen, the excess of radiations of long wavelengths is cut off and a spectral distribution closely approximating daylight is obtained.

Colored illumination may sometimes be useful in visual microscopy, particularly in the study of colored objects. In general, an object which is seen chiefly as a color image will appear dark and opaque if illuminated with light of a complementary color, and pale and transparent if illuminated by light of its own color. Differently colored constituents in mixtures, such as dyed textile fibers, stained paper fibers, or food materials, may be accentuated by the use of light of the proper color.[44] The most suitable hue may need to be determined by trial, and may not be the same for photographic contrast as for visual contrast. Colored light may be advantageously employed in dark field illumination of colored objects, or by the method of differential color illumination.

The use of approximately monochromatic light to minimize the effect of chromatic aberration is sometimes advantageous. For this purpose a green filter is usually chosen, since objectives are best corrected for this color. Determinations of refractive index and other optical properties are rendered more accurate by the use of color filters giving approximately monochromatic illumination (page 374). Blue or violet light is used to give increased resolution (page 11).

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[43] Manufactured by the Corning Glass Works, Corning, N. Y., and described by Gage: *The Microscope* (1925), pp. 48–53.

[44] The colored filters and plates in the pamphlet on *Photomicrography* (Eastman Kodak Co., 1927), illustrate this phenomenon very strikingly.

Light of the color desired may be obtained by means of a color screen or filter placed in front of the light source. Such filters are either colored gelatin mounted between glass,[45] or solid glass plates of suitable color.[46] Solutions of dyes may also be used.[47] None of these color filters gives strictly monochromatic light, but they transmit an appreciable range of wavelengths.[48]

Certain filters are made for the purpose of cutting off all but a single bright line of the spectrum of the mercury arc, so as to give strictly monochromatic green, blue, or violet light. Unfortunately, high intensities are not obtainable by such means, since only a small fraction of the total radiation is utilized.

A simple device for obtaining approximately monochromatic light of any wave length may be improvised by means of a replica diffraction grating (14,000 to 25,000 lines per inch), mounted on the microscope mirror. Parallel rays should be supplied by means of an auxiliary condenser, and the substage condenser should be focused on the object. The color of the illumination may be varied by tilting the mirror. Since adjacent portions of the spectrum appear imaged in the field, all but the desired hue should be cut off by means of a field diaphragm at the light source. A hollow prism filled with carbon bisulphide may be arranged to produce a spectrum from an arc lamp which furnishes parallel rays. By placing the microscope in the proper position, any portion of this spectrum may be made to fall upon the mirror.[49]

A direct vision spectroscope with wavelength scale, or a spectroscopic eyepiece, is convenient for determining the wavelength of the light selected for the microscopic observation.

Monochromators, constructed on the principle of the spectroscope, may be used where a very narrow spectral region is required.[50] However, the light which they supply is not strictly monochromatic unless the apertures of the monochromator are reduced, and this involves a serious loss of intensity. This disadvantage and their high cost limit the usefulness of such instruments to special investigations. For ordinary work, including the study of optical phenomena and refractive indices of crystals, the simpler devices described above furnish illumination which is sufficiently monochromatic.

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[45] Wratten Filters, obtainable from the Eastman Kodak Co., Rochester, N. Y. Ilford Filters, obtainable from W. Watson & Sons, London.

[46] Chance-Watson Filters, obtainable from W. Watson & Sons. The spectral transmissions of a number of colored glasses made by the Corning Glass Works are described in U. S. Bur. Standards *Technologic Paper* **148** (1920). Several of these give approximately monochromatic light, and are suitable for use in microscopy.

[47] The transmission curves of a number of suitable solutions are given by Holmes: *Amer. Dyestuff Rept.* **17**, 31 (1928).

[48] The " Monla " lamp of Leitz is designed to be used with special filters, and at intensities which are governed by a rheostat and ammeter, so as to give light approximating the wavelength of either the C, D or F lines of the spectrum, within a range of ±15 $m\mu$.

[49] A number of other simple methods of obtaining monochromatic light are described by Wood: *Physical Optics* (1919), pp. 11–17.

[50] Manufactured by Bausch & Lomb, Leitz, and Fuess.

A special monochromator is used as a part of ultraviolet photomicrographic apparatus, to isolate the intense lines (275 mμ) of the cadmium spark, which are necessary for extreme resolution with quartz "monochromat" objectives.

In photomicrography the **actinic character** of the illumination is highly important. With ordinary emulsions, only the shorter wavelengths of light function to produce the photochemical image. The effect is similar to the use of a color filter transmitting only blue, violet, and ultraviolet. If the light source is rich in these radiations, it will be satisfactory for photographic purposes; if not, the *effective* illumination will be of low intensity. Even if the photographic emulsion is rendered sensitive to all wavelengths, the blue and violet are disproportionately powerful, and must be decreased somewhat by a complementary yellow filter if the relative brightness of different colored objects is to be truly rendered. The "color-screen" effect of the photographic emulsion must be neutralized to varying degrees, depending on its sensitivity to different colors of light (orthochromatic or panchromatic character).[51] If, in addition, it is desired to obtain increased contrast or transparency, as described above, the actinic qualities of the various color filters introduce complications. Unless plates equally sensitive to all colors are used, visual observation of color effects may be rather misleading. Actual trial of the color filters suggested by the foregoing considerations is the simplest method of obtaining satisfactory rendition of values.

On account of the high intensity of blue and violet in the arc, even more allowance must be made for the non-uniform sensitivity of the photographic emulsion than in the case of illumination by incandescent lamps, and a strongly yellow filter may be necessary. Compensating filters are unnecessary for photography of colorless objects, however, so the full actinic efficiency of the arc may be utilized in most photomicrography. The arc possesses several times the actinic power of the various incandescent lamps, of which the ribbon-filament and tungsten are types rank highest.

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[51] "*The Photography of Colored Objects*," published by the Eastman Kodak Co., gives an excellent discussion of this and related problems.

The **manipulative convenience** of a source of light for microscopy should be such that a minimum of attention is required after the preliminary adjustments (which should be very simple) are made. Incandescent electric lamps are ideal in this respect, but modern automatic arc lamps also require very little care, if their mechanical construction has been well designed.

As a general purpose lamp that shown in Fig. 24 is particularly useful. It consists of a concentrated-filament, 6-volt, 24-watt tungsten bulb, one side frosted, in a sliding mount set in a cylindrical housing with a condensing lens. The lamp bulb may be moved from or toward the lens to give converging or parallel rays. It may be raised or lowered to insure proper centration in the axis of the condensing lens, and it may be turned frosted side forward to give a light source of large area. Two types of condensing lenses, "spherical" and "aspherical," are obtainable; the latter is preferable, as having less spherical aberrations and a higher effective light-grasping power. A plate of "Daylite" glass inserted behind the lens will add greatly to the usefulness of the lamp; a slot may be cut in the top of the housing, so that it may be readily removed, or other color filters substituted.

The vertical support rod should be at least 30 cm. in height, to permit the illumination of objects by inclined reflected light. The double clamps permit raising and lowering or tilting the lamp in any direction, and render it useful for the illumination of scales and apparatus other than microscopes. Its intensity is sufficient for most dark field illumination, and even for photomicrography at moderate powers. A 6-volt stepdown transformer is necessary in order that it may be attached to the 110-volt A-C. lighting circuit. The chief defect is a tendency for the contacts of the lamp base and socket to deteriorate, on account of the heavy current.

The "convertible substage lamp" of Bausch & Lomb also operates at 6 volts. The light source is a small incandescent bulb with V-type filament. The housing (Fig. 54) is very light and compact, so that it may be attached directly to a substage condenser, dark field illuminator, or vertical illuminator, or may be mounted on a support stand for inclined illumination of opaque objects. Its chief advantage is in connection with a vertical illuminator, for the microscope may be focused freely without destroying the alignment of the illumination, as would be the case if the light did not move with the illuminator.

Larger incandescent lamps, which work directly at 110 volts, are obtainable from a number of manufacturers. These usually are mounted on an inclinable stand, and have a focusing condensing lens. The lamp bulb should contain a concentrated spiral filament, preferably arranged in one plane (Fig. 43, B) so that it may be imaged as a unit in the aperature of the substage condenser or in the plane of the preparation, and its image rendered uniform by throwing slightly out of focus. Lamp bulbs of this type are commonly sold for use in projection lanterns; the 200- to 500-watt sizes are ordinarily sufficient for photomicrography. Housings should be well ventilated, but should prevent the escape of stray light. They may be improvised if desired, an auxiliary condensing lens being mounted separately.

The lamp shown in Fig. 43 is especially well designed to provide adequate illumination for critical microscopy, dark field studies, and photomicrography. It is equipped with a two-lens condenser, in front of which is an iris diaphragm; by altering the position of the bulb parallel or convergent rays may be obtained, and a disk of Daylite glass may be inserted for visual work. This lamp comprises the optical system necessary for Köhler's method of illumination, and is equally well adapted to other procedures.

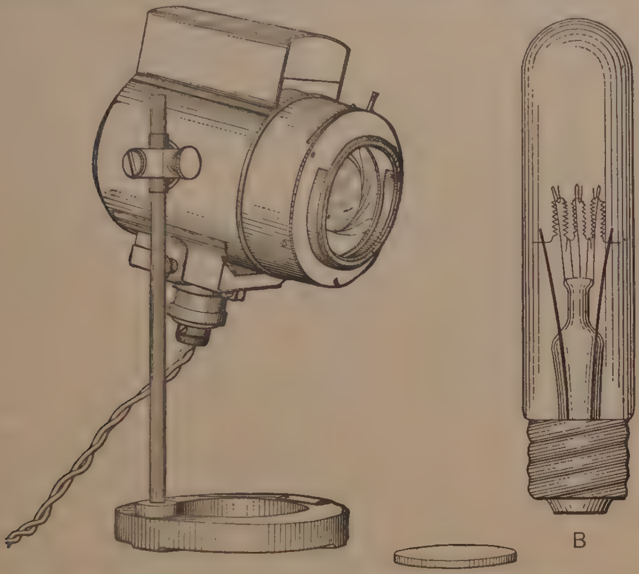

FIG. 43. Microscope Lamp with Condenser and Diaphragm (Spencer Lens Co.).

Incandescent lamps with spiral filaments are open to the objection that the light source, although it covers a considerable area, is far from uniform. This is not important, if Köhler's method of illumination is used, but is undesirable if the filament is actually imaged in the plane of the object. To avoid this difficulty ribbon filament lamps, working at 6 volts and 108 watts, have been placed on the market.[52] The filament consists of a single strip of tungsten, about 2 mm. broad, and constitutes a homogeneous light source of higher intensity than a 110-volt lamp.

Tungsten arc lamps supply light of high intensity and actinic value.[53]

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[52] By Bausch & Lomb, Zeiss, and Leitz.

[53] " Pointolite " lamps are obtainable from Zeiss, or from any of the English microscope dealers.

The radiant is a sphere of tungsten enclosed in a glass bulb containing an inert gas. An auxiliary starting resistance is necessary; either direct or alternating current may be used.

Arc lamps of various types constitute the most powerful source of light commonly used by the microscopist.[54] The intrinsic brilliancy varies, being lowest with alternating-current arcs, higher with direct-current arcs, and still higher if special metal-covered carbons are used. However, for any type of carbon arc light, the brightness per unit radiant area is practically unaffected by the quantity of current consumed. The principal advantage of using heavy currents is that a larger crater is formed, and the area of maximum brightness is increased. This may be highly desirable in photomicrography and projection, because the larger the light source the less it need be magnified (and thereby reduced in brilliancy) to fill the aperture of the microscope condenser.

Direct current is almost a requisite for an arc lamp; alternating current gives a poorer light, and causes an objectionable hum and flicker. By means of a suitable resistance in series with the arc, blown fuses are prevented when the arc is "struck," and the current is maintained at about 4 to 6 amperes for most work; microprojection may require 8 amperes or more, and for ultramicroscopic investigations an arc of 15 to 30 amperes is often desirable.

The positive electrode attains a higher temperature and greater brilliancy than the negative one; a crater forms in the carbon, and this is always utilized as the actual source of light. The best emission of radiation takes place if the carbons are arranged at right angles to each other, the positive one horizontal with the negative one slightly in advance of it and lowered just sufficiently to avoid obstructing the path of light. Wandering and fluctuation of the crater are minimized by the use of carbons with soft cores; small-diameter carbons are also used for this purpose. Copper-coated carbons have high conductivity and emissivity, resulting in a light of extreme intensity, if very heavy currents are employed. Both carbons are gradually consumed in the arc; the positive one burns more rapidly, and is usually made larger for this reason. The rate of burning is greater the farther the carbons are separated.

Hand-feed arc lamps are inexpensive, and permit the use of carbons of various sizes and currents of various strengths. Provision is made for independent adjustment of each carbon, usually by means of coaxial screws (Fig. 44). The intensity of illumination of hand-feed arcs varies considerably, depending on the length of the arc as the carbons gradually burn away; the position of the crater also is likely to shift. Rather frequent "trimming" is therefore necessary, and may be a considerable inconvenience if it interrupts important visual observations.

Automatic-feed arc lamps are considerably more expensive than those fed by hand; in most models each carbon must be of a certain size if the rate of feed is to keep up with their burning away. Both carbons are moved by the same mechanism and may not be readily adjustible independently of each other. The feed mechanism is the most fallible part of any automatic arc lamp, for it must operate at a high temperature, often for periods of several hours, with smooth and uninterrupted movement. The carbons must be maintained in their proper relative positions, if possible until they are consumed. This last, however, is hardly attainable, since the rate of burning is much greater with a long arc than with a short one, and the user must adjust the speed regulator of the mechanism to correspond with the rate at which the carbons are used up, in each case. Continuous feed by clockwork is preferable to discontinuous feed by the periodic action of a solenoid, for the latter type is likely to cause fluctuation in the position of the crater, and may move the carbons just as a critical observation or a photomicrograph is being made.

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[54] A good discussion of arcs of various types is given by Gage: *Optic Projection* (1914), pp. 535–570.

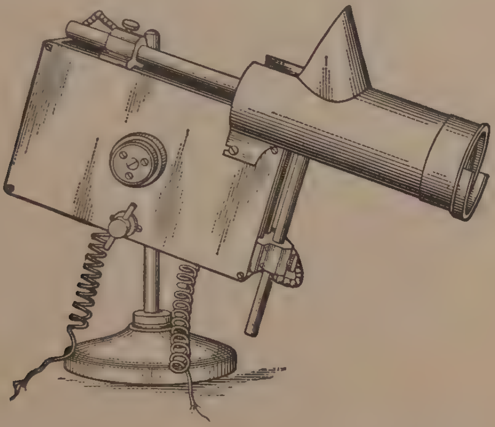

FIG. 44. Hand-feed Arc Lamp (Spencer Lens Co.)

A small automatic arc lamp of the type shown in Fig. 45[55] is very satisfactory in the above respects, and requires practically no attention in operation. In the opinion of the writers it is superior to the more recent automatic arc by the same maker. The housing is provided with a focusing condensing lens, which may be obtained in *Uviol* glass or quartz, if the maximum intensity of ultraviolet radiation is required. With appropriate resistances the same lamp may be used at either 5 or 8 amperes. The positive carbon is 8 mm. in diameter; the negative, 6 mm. Although the feed mechanism is designed for use with direct current only, it functions fairly well if alternating current is employed, both carbons in this case being 7 mm. in diameter. The writers have found this lamp almost indispensable as an all-purpose light source of high intensity and actinic value, suitable for photomicrography and projection at high magnifications, and for use with all types of ultramicroscopes.

Absorption of heat radiation is necessary whenever powerful light sources, particularly arcs, are employed for microscope illumination. Otherwise the substage condenser or the objective may be overheated and injured; a polarizing prism may be ruined by a few seconds' exposure to the concentrated beam of an arc lamp. The object under examination may be seriously affected by heat, and accurate observations of thermal phenomena by means of a hot or cold stage may be rendered impossible. In photomicrography at very high magnifications the heat from the light source may cause unequal expansion of the apparatus, resulting in a serious shift of focus. Until recently cooling chambers filled with water have been the best (but not very effective) means of absorbing the heat emitted by the lamp and concentrated by the auxiliary condenser.

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[55] Obtainable from Leitz. Spencer Lens Co., and Bausch & Lomb manufacture similar lamps.

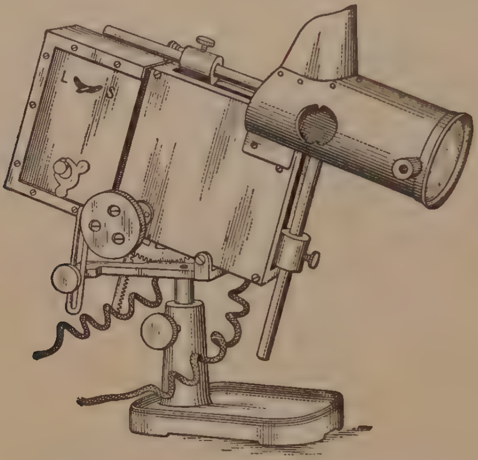

Fig. 45. Automatic Arc Lamp with Clockwork Feed (Leitz).

A **heat-absorbing glass**[56] has recently been perfected, which is far superior to a water cell. It obviates all risk of overheating apparatus or object, and its pale green color is hardly noticeable even when colored objects are illuminated. Various shades are obtainable, of which the lighter ones are preferable.

The temperature of the glass is rapidly raised by the heat which it absorbs and breakage is likely to result from uneven heating. To avoid this, it is advisable to immerse the glass in water in a small cell, to equalize its temperature.[57]

[56] "*Aklo.*" Manufactured by the Corning Glass Works, Corning, N. Y. It is said to stop 80 per cent of the heat radiation with only about 10 per cent loss of light.

H. P. Gage: *Tr. Soc. Motion Picture Eng.* **12**, 1063 (1928)

[57] Suggested by Professor S. H. Gage.

CHAPTER IV

ILLUMINATION OF OPAQUE OBJECTS METALLOGRAPHIC MICROSCOPES

Although the reflecting properties of opaque objects are necessarily the basis of their microscopic visibility, the methods of examination by reflected light are also frequently applicable to relatively transparent materials. The apparatus and technique employed are essentially the same, and the interpretation of appearances follows similar rules. Many striking and instructive analogies exist between the phenomena of reflected and transmitted illumination, and between the optical principles of the types of illuminating apparatus used. Practice in the illumination and study of transparent objects is a valuable preliminary training for work with reflected light, and the reader is encouraged to familiarize himself with the general discussion given in Chapter III before proceeding to the examination of opaque objects by more specialized methods.

Interpretation of appearances by reflected light depends almost wholly upon the application of the laws of reflection — in particular, that the angle of reflection of light from a surface is equal to the angle of incidence. The observer is of course not concerned with these angles *per se* but because the positions of the various reflecting surfaces of the object are thereby indicated. The pattern of light and dark which constitutes the reflection image seen in the microscope depends upon the arrangement of the minute surfaces of the object with reference to the illumination and the objective, and upon the inherent reflecting power of these surfaces. They will appear bright whenever they are oriented so as to reflect light within the angular cone of the objective, and dark whenever this is not the case.

The proportion of incident light reflected from a surface in a given position depends upon the "coefficient of reflection" of the material, which may be more than 90 per cent for silver, less than 60 per cent for iron, and less than 5 per cent for glass, at perpendicular incidence. The reflecting power may vary for light of different wavelengths (selective reflection) so that the reflected light is colored, as in the case of copper or brass. Differences of reflecting power (analogous to differences of light absorption) may give a **color image** by reflected light, as in the case of polished, unetched α–β brasses, α–δ bronzes, white cast iron, or aggregates of differently colored ore minerals.

In the case of specimens of uniform coefficient of reflection, structure is revealed by **reflection images** only. Like refraction images of transparent objects, these vary widely depending on the character of illumination. A plane mirror surface S (Fig. 46) such as that of a polished, unetched metal, lying in a plane normal to the axis of the microscope and illuminated by an inclined beam *a*, will reflect light specularly in the direction of *b*. If the angle of incidence is greater than half the angular aperture of the objective, the angle of reflection will be such that no directly reflected rays can be included, and no light will pass through the microscope. Consequently, the surface will appear *dark*, and the more perfectly it reflects, the darker it will be. If the angle of incidence is decreased to less than half the angular aperture of the objective, the illuminating beam *c* will be reflected in a direction *f* which comes within the angular cone of the objective, and the surface will appear *bright*. These two extremes of dark and light appearance depend on the obliquity of illumination, just as in the case of dark field and bright field illumination (pages 43, 86), and the angle of demarcation between them is indicated by *c d*, the angular aperture of the objective.

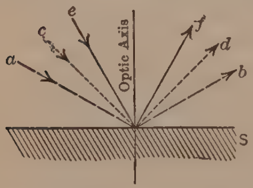

Fig. 46. Paths of Inclined Rays striking a Plane Polished Surface.

If the surface of the object illuminated by the inclined beam is roughened, scratched, or irregular in places, the irregular areas will reflect less specularly than the smooth portions, and will scatter light in many different directions. As a consequence, they will appear *light* with inclined illumination from outside the angular cone of the objective, and *dark* if the incident light comes within this cone.

Scratches, etching pits, and other uneven places thus appear dark against the bright background of the smooth areas, or vice versa, according as the angle of incidence of the illumination is varied, just as similar irregularities in transparent objects may appear dark or light with bright field or dark field illumination respectively.

Fine structures, near the limit of the resolving power of the microscope, are sometimes very difficult to interpret on account of diffraction patterns and other anomalous effects. The greatest care must be taken to focus very exactly, in order that spurious images may not give rise to false conclusions.

Specimens which possess a great many minute reflecting surfaces " scatter " light in all directions, and appear bright whatever the direction of illumination. The pigment granules in paints and enamels, the mineral grains of porcelain, the surfaces of fibers in paper, and similar fine structures, often permit the examination of the specimen by the light which they " reflect " diffusely, under inclined illumination. Specular reflection is at a minimum and a large proportion of the scattered light is included within the angular cone of the objective.

Elevations and depressions in opaque materials appear under the microscope much as in ordinary macroscopic observation; shadows are cast, and high lights produced in a similar manner. However, it must be borne in mind that directions are reversed in the microscope, and a shadow cast by an elevation on the side away from the illuminating beam will appear on the side toward it. This may lead to depressions being interpreted as elevations, and vice versa, unless the observer is careful to make his judgments on the basis of the actual, rather than the apparent positions of light and shade. Use of the fine adjustment as a means of obtaining " optical sections " (page 177) and of the Greenough (erecting) binocular microscope for moderate magnifications, are useful checks on any questionable appearances.

Applications of Studies by Reflected Light. — The extraordinary interest in the microscopic study of metals and alloys, which has largely developed within the last twenty years, has attracted attention to methods and equipment for the study of opaque materials. Microscopes and illuminating apparatus are being constantly improved, in response to the increasing demand for instruments which are precise and easily adaptable to a variety of purposes.

By far the largest use of such microscopes is in the study of the structures of metals — metallography. This science is rapidly extending more and more into the province of the chemist, and its findings are of considerable importance in all fields of investigation having to deal with solids and phase changes.

The special technique of metallography is given in many books, which are essential as a supplement to the present chapter.[1]

Many other substances are studied by similar methods: coal, concrete, cement clinker, ceramics, natural and artificial stones, textiles, paints, enamels, glazes, refractories, carbon electrodes, abrasives, resins, slags, mattes, and numerous other materials and products of chemical industries. Abrasion, polishing, corrosion and other surface phenomena may be observed, and in general any substance which is not conveniently examined by transmitted light is an appropriate subject for this procedure. Metallographic methods and apparatus are being widely employed in the study of opaque minerals,[2] and similar methods are applicable to other nonmetallic objects. Biologists are beginning to use reflected light in examining animal and vegetable tissues[3] even though these may be somewhat transparent, and therefore capable of being studied by transmitted light as well. Suggestions valuable in the investigation of novel materials may be gained from the work in the various fields mentioned.

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[1] Desch: *Metallography* (Longmans, Green, & Co., New York, 1922).

Sauveur: *Metallography and Heat Treatment of Iron and Steel* (McGraw-Hill Book Co., New York, 1926).

Hoyt: *Metallography* (McGraw-Hill Book Co., New York, 1920).

Williams and Homerberg: *Principles of Metallography* (McGraw-Hill Book Co., New York, 1928).

Guillet and Portevin: *Précis de Métallographie Microscopique et de Macrographie* (Paris, 1924).

Goerens: *Einführung in die Metallographie* (Halle, 1926).

[2] Campbell: The Microscopic Examination of Opaque Minerals. *Econ. Geol.* **1**, 751 (1906).

Ray: The Reflecting Microscope in Mining Geology and Metallurgy. *Min. Sci. Press* **108**, 922 (1914.)

Murdoch: *Microscopical Determination of the Opaque Minerals.* (John Wiley & Sons, New York, 1916.)

Whitehead: Notes on the Technique of Mineragraphy. *Econ. Geol.* **12**, 697 (1917).

Davy and Farnham: *Microscopic Examination of the Ore Minerals.* (McGraw-Hill Book Co., New York, 1920.)

Schneiderhöhn: *Anleitung zur Mikroskopische Bestimmung und Untersuchung von Erzen und Aufbereitungsprodukten im auffallenden Licht* (Berlin, 1922).

Fairbanks: *Laboratory Investigation of Ores.* A symposium. (McGraw-Hill Book Co., New York, 1928.)

See also the references given on page 162.

METHODS OF ILLUMINATION BY REFLECTED LIGHT

In general an opaque object is arranged before the microscope with the surface to be studied practically perpendicular to the axis of the objective, in order that the entire field may be visible instead of slanting out of focus. The illumination may be unidirectional, incident at any angle, in any azimuth, annular, or convergent. Unlike illumination of transparent materials, a single condenser is incapable of furnishing all these various types of illumination, and a number of different **opaque illuminators** have been devised for such purposes.[4]

Illumination by a direct beam from a light source is the simplest method (analogous to illumination of transparent objects by the mirror alone) and is very appropriate for large objects which are to be studied at low magnifications.

Axial illumination may be obtained by placing a reflector between the objective and the specimen, so as to send light practically parallel to the axis of the microscope and normal to the surface under examination. The reflector of necessity must be

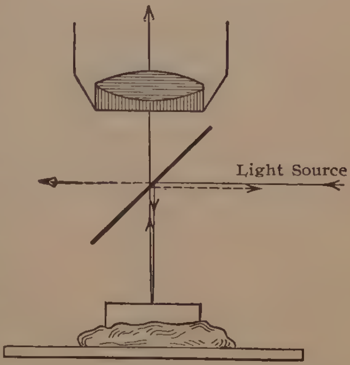

FIG. 47. Vertical Illumination by a Transparent Reflector between Objective and Specimen.

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[3] Schmidt: *Zeits. wiss. Mikros.* **37**, 101 (1920); *Mikrokosmos* **17**, 33 (1923–4).

Hauser: *Zeits. wiss. Mikros.* **42**, 280 (1925).

Vonwiller: Abderhalden's *Handbuch der biologischen Arbeitsmethoden*, Abt. V, Tl. 2, Hfte. 10, pp. 1033–54; *Protoplasma* **1**, 177 (1927).

Becher: *Zeits. wiss. Mikros.* **46**, 89–124 (1929).

[4] See Guthrie: Use of Various kinds of Light in Metallography. *Trans. Am. Soc. Steel Treat.* **3**, 710, 757 (1923).

transparent, in order that it shall not prevent light from going to the objective to form an image in the microscope. A piece of smooth clean glass supported by a clamp or by a special holder at an angle of 45°, and a light source (preferably with auxiliary condenser) are easily adjusted to give the desired illumination (Fig. 47). A piece of ground glass or tissue paper may be placed in the path of the light to the reflector, to render it more diffuse, as an aid in lighting up shadows and depressions. Obviously, external reflectors cannot be used with objectives of short working distance.

Inclined illumination (FO, Fig. 33) may be obtained by moving the above transparent reflector somewhat to one side of the axis of the microscope (analogous to swinging the mirror), altering its slope or the position of the light source if necessary. This procedure is suitable if very large fields must be illuminated, at almost normal incidence. However, it is usually unnecessary, and a direct beam from the light source is commonly employed. This may be most conveniently obtained by a lamp arranged as in Fig. 24. The angle of incidence may vary from just outside the angular cone of the objective to grazing or horizontal. By means of an auxiliary condenser (separate, or attached to the lamp) the light may be concentrated upon the object. Ground glass may be used as a diffusing screen, if sharp shadows and glaring highlights are undesirable. Two or more beams of light, axial or inclined, may be directed simultaneously upon an irregular specimen, to insure adequate illumination of all its surfaces. Light from a window falling upon the stage of the microscope will supply diffuse inclined illumination which is satisfactory for most visual work at low magnification.

The working distance of the objective sets a definite limitation upon the direction of inclined illumination, for if the front lens has to be placed very close to the object only almost grazing light can be supplied. Daylight illumination is practically impossible under these conditions, and artificial light sources serve little better. All unevennesses in the surface of the object are over-emphasized, and diffraction patterns are unduly prominent. Ordinarily, objectives of focal length much less than 16 mm. (or working distance less than 2 mm.) preclude good results with inclined illumination by the above methods, and require the use of some form of special illuminating device.

Inclined Illumination by Special Illuminators. — Inclined illumination at high powers is made possible by several types of reflecting illuminators, all of which send light to the object at an angle greater than half the angular aperture of the objective. Consequently polished surfaces appear dark, and rough areas, light. Any of these illuminators are applicable to the examination of transparent as well as opaque objects.

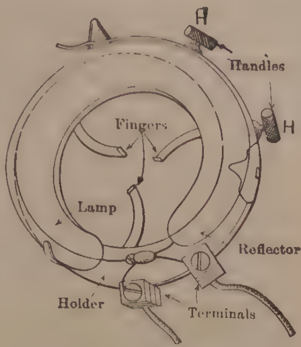

FIG. 48. Silverman Illuminator. Lamp and Holder.

Annular Illuminators. — The oldest and simplest of such illuminating devices is the "Lieberkuhn" reflector, an annular concave metal mirror which fits around the objective. If the specimen is mounted on a transparent slide, and light is sent upward past it, the light will be reflected downward by the annular reflector and converged upon the object in a hollow cone. The Beck "aplanatic ring illuminator"[5] consists of a glass ring ground to special curves and silvered on the upper surface, so as to give highly perfect annular illumination. It may be used with objectives of very short working distance. A special model is constructed integral with the mounting of a 3-mm. immersion objective, to give almost horizontal annular illumination.

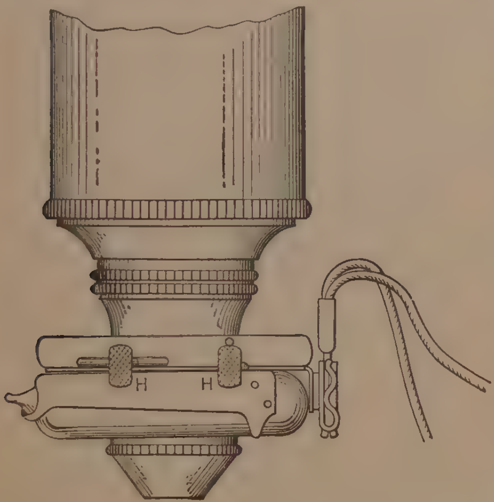

FIG. 49. The Silverman Illuminator attached to the Objective of the Microscope.

The Silverman illuminator[6] consists of a single-filament tubular tungsten lamp curved in the form of a circle (Fig. 48). The lamp is held in an annular mounting provided with three curved fingers under spring tension, which serve to hold the lamp on the objective, and may be opened by pressing the knurled heads HH (Fig. 49). The bulb may be obtained in colorless or "Daylite" glass. A resistance is necessary if it is used on 110-volt circuits.

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[5] R. & J. Beck, Ltd., London.

[6] Silverman: *Ind. Eng. Chem.* **9**, 971 (1917); **12**, 1200 (1920); *Met. Chem. Eng.* **18**, 318 (1918).

The illuminator is easily attached to the microscope, and is not thrown out of alignment by focusing. It may be supported by means of a clamp attached to the stage, for use with a Greenough binocular microscope. Annular illumination is obtained, the inclination of which may be varied by raising or lowering the illuminator. With objectives of short working distance only highly oblique illumination is possible. Part of the bulb may be screened by a shield, to give unilateral illumination and permit better accentuation of the shadows, which are almost lacking with annular illumination from all sides.[7]

The well-known property of curved transparent materials, which " conduct " light by total internal reflection, has been applied in various illuminators, such as that of Silverman[8] which utilizes rods of quartz glass. One end is placed close to the light source, the other near the object. Light travels along the rod and is emitted at the end, to give inclined illumination. Several rods may be used, to furnish illumination from more than one side.

The light from the above annular illuminators converges upon the object in a hollow cone, the angular aperture of which is entirely outside that of the objective.[9] As a result, no directly reflected rays are sent to the objective from smooth surfaces perpendicular to the axis of the microscope, and only fissures, edges, and rough areas appear bright. Unilateral inclined illumination may be obtained at fairly high powers, by means of annular illuminators of which only a sector is functioning. Special objectives with self-contained reflectors have been designed by Chapman and Aldridge[10] for use at high magnifications.

All of the methods of inclined illumination described above give appearances which are practically the negatives of those obtained with vertical illuminators. Roughness and irregularities are emphasized, and shadows from elevations or depressions are prominent, unless the illumination is from all sides. The user must learn to interpret appearances on a new basis, just as in the case of dark field illumination of transparent objects. The greatest usefulness of these illuminators appears to be in the study of irregular, poorly reflecting surfaces at moderate powers, though the special illuminating objective of Beck may be of value in interpreting metal structures at high magnifications.

Illumination by means of Vertical Illuminators. — Vertical, (or opaque) illuminators consist of a mounting containing some sort of reflector which sends light *through the objective* to the specimen but does not seriously obstruct its passage to the image. The objective functions as a condenser to illuminate the object, and also forms a real image of it in the microscope. Short focal lengths and working distances set no limitations on the use of vertical illuminators.

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[7] A modified illuminator is described by Silverman: *Ind. Eng. Chem.* **17**, 43 (1925).

[8] *Ind. Eng. Chem.* **17**, 573 (1925).

[9] This is analogous to dark field illumination of transparent objects (page 43).

[10] Described by Greaves and Wrighton: *Practical Microscopical Metallography* (1924), p. 16.

The types of illumination obtainable by the use of vertical illuminators can be understood only if the objective is considered as a condenser, the reflector being analogous to the mirror as used in transmitted illumination. At present, objectives for use with vertical illuminators are not equipped with diaphragms as are substage condensers, so the obliquity or convergence of the illumination must be controlled by external means.

The "**transparent reflector**" **vertical illuminator** (Fig. 50) is capable of supplying the greatest variety of illumination, the different types of which will be discussed in connection with it.[11] The illuminating rays I, I, enter through an opening O in the side of the mounting, and are reflected by the glass plate G to follow the path R toward the objective which is screwed in the lower end of the illuminator. The light not reflected by the glass plate (about 85 per cent) passes through it and is absorbed on the blackened walls of the mounting C. The initial intensity must be high to compensate for this loss.

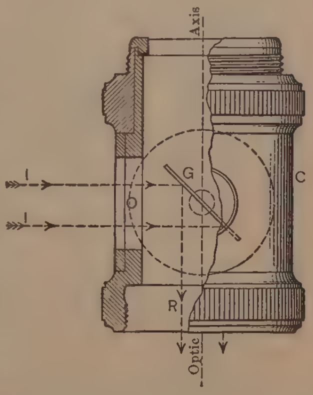

Fig. 50. Transparent Reflector Vertical Illuminator.

Since the glass plate reflector is usually almost as large as the back lens of the objective, the entire aperture may be filled with light. The objective can thus supply **symmetrically convergent illumination**, the angular cone being equal to its own angular aperture. The degree of convergence is regulated by narrowing the illuminating beam by means of a diaphragm outside the illuminator, so as to give strictly **axial illumination** instead of convergent illumination, if desired. As in the case of transmitted illumination (page 83), convergent light gives maximum resolution but rather weak contrast, whereas axial light enhances shadings and gives a bolder but less detailed image. The diaphragm of the vertical illuminator should be so located that it actually functions as an aperture diaphragm; or if a part of an auxiliary illuminating system, this should also be true. By careful adjustment of the diaphragm, the visibility of the object may be greatly increased, with a minimum sacrifice of resolution. The diaphragm should never be opened so wide as to illuminate more than the area of the back lens of the objective, or "glare" and stray light will become very marked.

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[11] The Spencer Lens. Co. has manufactured a vertical illuminator in which the reflector consists of two 45° prisms, the oblique face of one being partially silvered before the other is cemented to it. This constitutes a semitransparent mirror, embedded in a cube of glass, and functions similarly to a glass plate reflector. However, the interposition of a cube of glass in the path of the image-forming rays from the objective introduces some optical difficulties as regards the corrections of the lenses.

Critical illumination is obtained when the source of light is imaged in the plane of the object. This might be done irrespective of its location, by moving the objective, but this would mean ignoring the focusing of the objective to form an image in the microscope. Actually, the objective is always focused on the preparation, and the position of the light source is adjusted so it will be imaged on the surface of the object. The distance of the light source from the objective must be equivalent to that of the real image in the microscope (in other words, to the optical tube length). If the entire field of the objective is to be illuminated, the area of the light source must be equivalent to the area of the real image formed in the eyepiece.[12]

Oblique illumination[13] by reflected light may be obtained by screening one side of the opening of the illuminator, or better, by decentering the illuminating beam by means of an excenterable aperture diaphragm. Since the maximum obliquity possible is still within the angular cone of the objective, bright field illumination always results.

Annular[14] or **epiphragmatic**[15] **illumination** is secured by inserting a central stop in the plane of the aperture diaphragm of the illuminator (*cf.* page 43). A hollow beam of light is supplied to the reflector, and a hollow cone of illumination results. However, this does not give rise to dark field illumination, for the most oblique rays are incident on the surface of the specimen at an angle equal to half the angular aperture of the objective, and are reflected at an equal angle, so they are all included within the angular cone of the objective.[16] Conical illumination gives greater contrast and appearance of relief than does convergent illumination, without the sacrifice of resolution which accompanies a narrow illuminating cone.[17]

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[12] Wrighton: *Jour. Roy. Micros. Soc.* **279**, 107 (1927), gives a good discussion of these factors.

See also Fig. 55.

[13] Portevin and Gartner: *Rev. Métal.* **7**, 921 (1910), were among the first to urge the use of oblique illumination in metallography.

[14] George: *Trans. Am. Soc. Steel Treat.* **4**, 140 (1923); *Trans. Am. Inst. Min. Met. Eng.* **70**, 259 (1924), has been the leading exponent of this method of illumination.

[15] The term "epiphragmatic illumination" was coined by Benedicks: *Metallographic Researches* (1926), p. 170.

The transparent reflector transmits only about 70 per cent of the intensity of the image-forming rays. However, the aperture of the objective is unrestricted, and its full cone is available for maximum resolving power. In conjunction with suitable auxiliary condensers and diaphragms (page 127) it permits all types of "bright field" illumination of opaque objects. Vertical illuminators of this type are particularly suited to work at high magnifications. Their chief defect is the low efficiency of reflection of light, but a powerful light source and a highly reflecting object tend to counteract this.

The **mechanical construction** of transparent reflector vertical illuminators varies widely with different makers. The reflector should be easily adjusted to the proper angle, and readily removable for cleaning. The glass plate should be more than large enough to cover the entire back aperture of the objective, even when tilted at 45° to the axis of the microscope.[18] The surfaces of the glass should be plane and well polished; ordinary cover-glasses are not satisfactory. If an iris diaphragm is a part of the illuminator, it should be as close to the reflector as possible, in order not to function as a field diaphragm. The housing and all interior parts of the illuminator should be thoroughly blackened, to absorb all stray light.

The **adjustment** of a vertical illuminator of the type described above involves, as a preliminary, arranging the light source to send rays into the side opening, tilting the reflector to send light downward, and focusing the microscope on the object, which should preferably be rather strongly reflecting. These steps may be facilitated by looking *into* the illuminator as well as *through* it, and in particular by observing it in the body-tube with the eyepiece removed. The relationship between objective aperture and illuminating aperture is readily seen by this method.

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[16] This is analogous to a substage condenser and central stop, used in connection with an objective having an angular aperture greater than the dark cone — in fact, as great as the condenser itself.

[17] Benedicks made a careful study of conical illumination and gives many excellent illustrations of the results obtained with it. *Op. cit.* Chap. V.

[18] Both Leitz and Watson & Sons manufacture illuminators specially designed in this respect.

The quality of the illumination is then perfected by making sure that the light enters horizontally, and in a direction perpendicular to the axis of rotation of the glass reflector. The light source may need to be moved to affect this alignment. The distance of the light source should be adjusted so that it is imaged by the objective on the surface of the object. Some illuminators are equipped with a lens sliding in a side tube, as an aid (rather ineffective) to this adjustment. The size of the light source may well be restricted by a perforated screen, unless an auxiliary condenser and diaphragm are used as a *secondary* light source, as is the case in most large metallographic microscopes. In either case, the diaphragm at the light source should be closed until it just fails to restrict the field of the objective.

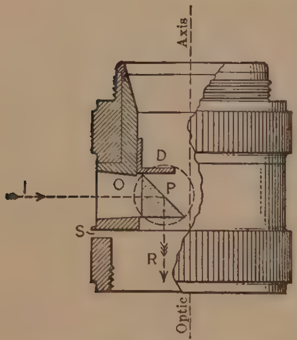

FIG. 51. Prism Vertical Illuminator.

The aperture diaphragm of the illuminator should be closed sufficiently to cut off any light from the reflector frame or other parts of the mounting. If this diaphragm is mounted separately, as in some large metallographic microscopes, it should be reduced until it supplies a narrow beam which strikes only the reflector, and is not scattered by encountering the edges of the side opening of the illuminator.

Prism vertical illuminators (Fig. 51) are capable of supplying a very high intensity of illumination, but this is necessarily more or less oblique. The illuminating beam *I* enters the opening *O* in the side of the mounting, is reflected by the 45° totally reflecting prism *P*, and follows the path *R* to the objective. As an illuminating system, this is analogous to the use of the substage condenser with all but a segment of its aperture covered by a screen. The rays pass through the edge of the objective, and are refracted to give unilateral oblique illumination (GO, Fig. 33). The maximum obliquity is equal to half the angular aperture of the objective, and the specularly reflected light from the object is utilized to form an image as in bright field illumination. Similar but less intense illumination may be obtained by means of a transparent reflector, only one edge of which is illuminated (page 118).

The brilliant oblique illumination obtainable from a prism vertical illuminator is especially useful in connection with examinations of poorly reflecting substances, or in photomicrography with light sources of only moderate intensity. Shading is somewhat unsymmetrical, so that an appearance of " relief " is gained, and elevations or depressions appear contrasted and realistic in the image. Since the full aperture of the objective (acting as a condenser) is not utilized for illumination, maximum resolution is not possible by the use of illumination of this type.

As an image-forming system, the aperture of the objective is also restricted by the prism interposed in the path of the light from the object. Although the prism screens a segment less than half the area of the back lens, a definite loss of resolution results[19] particularly for structures parallel to the edge of the prism. Symmetrically convergent or " critical " illumination is impossible with prism illuminators, as is also annular illumination. The larger metallographic microscopes are generally arranged so that the prism may be easily replaced by a transparent reflector, for examinations requiring maximum resolution.

Adjustment of prism illuminators involves little more than directing a beam of light at the prism which has been tilted to the proper angle. The illuminating beam should be no larger than the width of the prism, and should be centered on its vertical face. The prism should be tilted so that the image of the aperture diaphragm is visible in the middle of the unobstructed half of the objective aperture, as observed on removing the eyepiece and looking down the draw-tube.

Mirror vertical illuminators reflect light to the aperture of the objective by means of a small movable mirror. The mirror may be semicircular in shape, in which case it functions exactly as does the prism in the illuminators just described, furnishing unilateral oblique illumination.[20]

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[19] Benedicks: *Metallurgie* **6**, 320 (1909). The illuminator of the large metallographic microscope of Zeiss is equipped with a prism which covers a *sector* instead of a segment of the aperture of the objective. This renders more of the aperture available for resolution.

Tiny circular mirrors are commonly employed for illumination at moderate magnifications (Fig. 52). These supply a narrow beam of light to the objective (analogous to a nearly closed diaphragm with a substage condenser) and may be moved laterally across its aperture (like an excenterable substage diaphragm) to give unidirectional illumination, either axial or oblique. The illuminating cone is necessarily small, but the aperture of the objective for image formation is not seriously reduced. Such illuminators furnish intense light, readily variable to different degrees of obliquity within the angular aperture of the objective, and are very useful in bringing out three-dimensional structures in the specimen. They are easily adjusted by trial, the chief precaution being that the beam of light supplied from the side should be narrow, and well centered on the mirror, in order to minimize stray light.

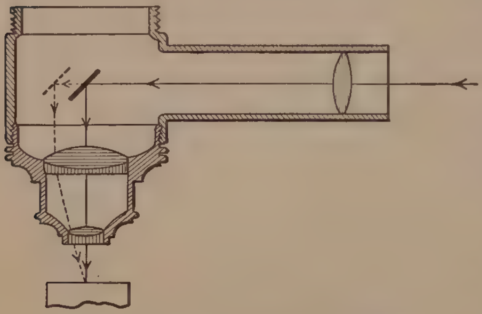

FIG. 52. Mirror Vertical Illuminator.

Objectives for use with vertical illuminators commonly have short mountings extending as little as possible beyond the back lens (Fig. 52). This permits the reflector of the vertical illuminator to be placed very close to the lens surface. By this means internal reflections are somewhat reduced, but the principal advantage is in connection with mirror or prism illuminators. In order that these reflectors shall not cast shadows on the field of the microscope, they must be as near as possible to the position where an aperture diaphragm would be placed, that is, at or within the back focal distance of the objective. If ordinary objectives are used the reflector is much farther away, and nearer the location of the field diaphragm in the image plane, so the field cannot be uniformly illuminated on account of its shadow. With illuminators of the transparent reflector type, no shadow can be cast by the reflector, and longer objective mounts are permissible.

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[20] Jewell: *Jour. Optical Soc. Amer.* **14**, 159–67 (1927), has devised an illuminator with a stellite mirror, the inner edge of which has an elliptical notch. Its illuminating rays make up half a hollow cone, and it is said to minimize glare from internal reflections.

R. & J. Beck manufacture a vertical illuminator which permits the widest possible variation of illumination. It may be used with either transparent or prism reflectors, or with mirrors of a variety of shapes and either plane or curved surfaces. The tilting of the reflector may be accurately controlled in two directions, and it may be laterally displaced in two directions.

Objectives of high aperture (>0.5) should be corrected for use with *uncovered objects*, and this is invariably done with short mount objectives unless otherwise ordered. The larger metallographic microscopes have body-tubes longer than 160 mm., in order to accommodate the prisms and mirrors necessary to their inverted construction. Consequently objectives for use with these instruments should be corrected for the tube length specified by the manufacturer, usually 200 to 215 mm. If these same objectives are to be used with an ordinary microscope, it should have a draw-tube which can be extended to this distance. The interior of objective mountings should be thoroughly blackened, to absorb stray light. This is properly a task for the manufacturer, though often neglected.

Cover-glasses should not be used with specimens to be studied by reflected light, not only because of the corrections of the special objectives commonly employed (page 25), but also because so much light may be reflected at the upper surface of the glass that the preparation beneath may be poorly illuminated, or veiled by this useless reflection. With homogeneous immersion objectives the conditions giving rise to these objections are absent.

“ Glare ” is a most serious hindrance to satisfactory illumination of opaque objects. It manifests itself as a haze of light which may be localized or may cover the entire field, obscuring shadows and destroying contrast. The effect of glare is many times more marked with vertical illumination than with illumination by transmitted light. If no object is in place, the field of the microscope ought to appear dark with vertical illumination, since no light is reflected back to the objective. Actually, as one can test by aligning the illumination and then removing the object, the field is far from dark, and may be more than one-tenth as bright as if a polished metal specimen were reflecting light back to the microscope. Such ever-present light is superposed on all shadows, and would never be tolerated in ordinary photography; it should not be accepted by microscopists and metallographers. Instruments differ greatly in this respect, and the user should test for himself their relative freedom from glare, and the conditions under which it is at a minimum.

A number of factors contribute to the stray light that is revealed as glare. The reflection of light from the first surface at the back of the objective may be very troublesome. In general, the more convex the outer surface of the back lens, the more the light reflected by it will diverge to the walls of the illuminator and body-tube and be harmlessly absorbed. Different objectives vary greatly in this respect. Further reflections from surfaces of other lens elements in the objective may account for a smaller part of the glare. Dust on lenses or reflector may scatter light, and bright brass interiors of objective, illuminator, or body-tube are very objectionable. If the illuminating beam encounters any bright surface such as the frame of the glass reflector or mirror, a considerable amount of light will be reflected up the body-tube. Proper centration of illumination will aid materially in reducing glare, as will reduction of the aperture of the illuminating beam. If a field diaphragm is a part of the illuminating system, closing it until only a small area in the center of the field is illuminated will cut down the glare very strikingly, and will give the user an idea of the clarity and definition which are unfortunately so lacking under ordinary conditions of vertical illumination.

The judicious use of the field diaphragm is one of the best methods of reducing glare. If the illuminated field is cut down to one-quarter of its original area, the intensity of light which enters the illuminator is proportionately reduced (page 48) and the part of this light which is reflected as glare is thereby lessened accordingly. The intensity of illumination over the reduced field is not decreased, and the contrast is therefore markedly improved, without any loss of resolution or brightness. In both visual and photographic work when large fields are not essential, it is often desirable to reduce the illuminated area to less than that of the visible field, for the study of fine details. Looking down the draw-tube may aid in tracing the origin of at least a portion of the stray light, and successive removal of specimen, objective, and reflector may indicate which of these is to blame. In general, prism or mirror illuminators appear to give less glare, though this may be due chiefly to the more intense illumination and more contrasty image which they produce.

Auxiliary Condensers and Light Sources for Vertical Illuminators. — The illuminator, with or without an attached iris diaphragm, can only serve to fill the aperture of the objective more or less completely, and so to affect the convergence or obliquity of the illumination. In order to regulate the area of the field illuminated, and to concentrate light in a narrow beam upon the opening of the illuminator, some form of auxiliary condenser

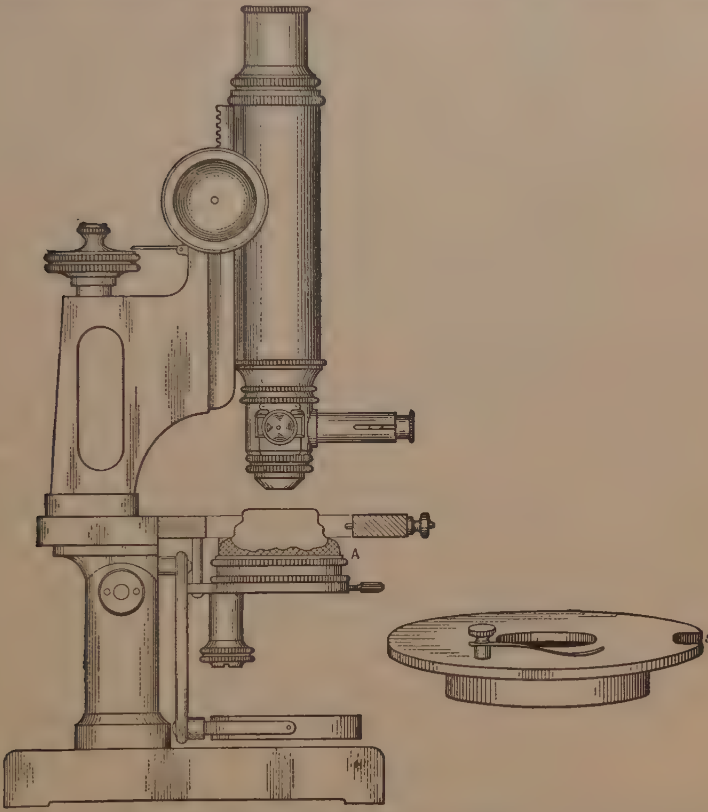

FIG. 53. Chemical Microscope with Stage removed and Auxiliary Stage inserted in the Substage Ring.

and diaphragm is desirable. The simplest arrangement may be merely a lens sliding in the side tube of the illuminator, which serves as an imperfect aid in obtaining critical illumination, with perhaps a diaphragm, which limits either field or aperture, or neither very definitely.

The less elaborate forms of vertical illuminators may be used in conjunction with a lamp such as is shown in Fig. 24; its condensing lens enables a concentrated beam of light to be supplied to the side opening of the illuminator. However, movement of the coarse adjustment in focusing the microscope destroys the alignment of this system, and a focusing stage (Fig. 56) is almost necessary.[21] The combination lamp shown in Fig. 54 obviates this difficulty, since it may be attached to the illuminator so as to remain in alignment irrespective of focusing movements.[22] It carries an auxiliary lens which gives approximately critical illumination, and will serve very satisfactorily to convert a "chemical" microscope for metallographic use.

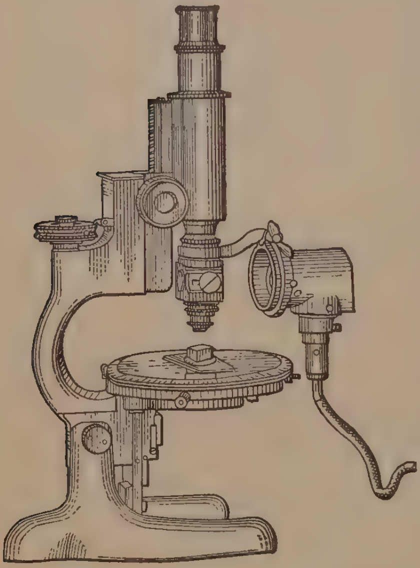

Fig. 54. Convertible Lamp mounted on the Microscope, for use with a Vertical Illuminator (Bausch & Lomb).

Regulation of both aperture and field requires a system similar to that diagrammed in Fig. 55, consisting of an aperture diaphragm *A*, in the side of the illuminator and a field diaphragm *F* at a distance equivalent to the tube length of the microscope. The light scource may be placed at this field diaphragm, but an enlarged image of it is usually formed there instead, in order that the full opening of the diaphragm may be filled with light. The field diaphragm F thus functions as a *secondary light source of variable size* (*cf.* page 98), and is imaged on the surface of the object when the objective is in focus. It is ordinarily opened just enough to cover the field of the microscope.

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[21] If the stage of a chemical microscope is removed, a cylinder of suitable dimensions may be placed in the substage ring, and used as a focusing stage (A, Fig. 53).

[22] Manufactured by Bausch & Lomb. Most firms list some similar arrangement of lamp attachable to the illuminator.

Instead of regulating the diameter of the illuminating beam by an aperture diaphragm in the side of the vertical illuminator, an external diaphragm may be used for this purpose. Such a diaphragm A is placed in front of the lens C which projects the image of the light source at F. An auxiliary condensing lens c, placed close to the field diaphragm F, images this aperture diaphragm A in the back focal plane of the objective A_1. By varying the opening of the diaphragm A, all or part of the aperture of the objective may be illuminated, and by the introduction of a screen or a central stop at this point, oblique or conical illumination may be obtained.

This is essentially the ideal arrangement discussed by Beck;[23] it is capable of a high degree of flexibility in furnishing the various types of illumination by reflected light. The proper adjustment of such a system of auxiliary condensers and diaphragms is more easily effected and maintained if some form of permanent support

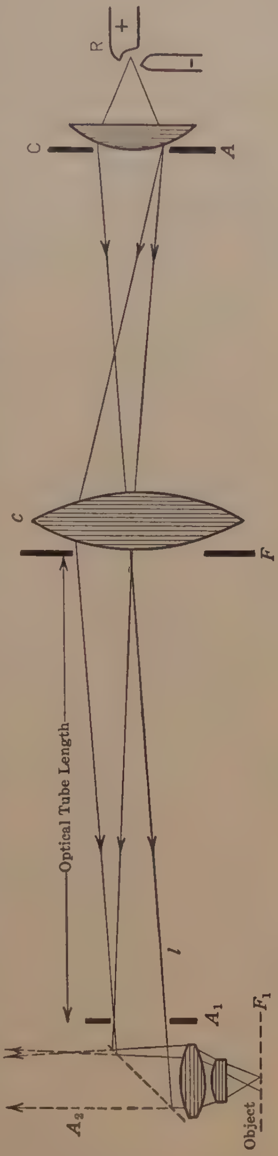

FIG. 55. Arrangement of Field and Aperture Diaphragms for use with Vertical Illumination.

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[23] *Jour. Roy. Micros. Soc.* **279**, 116 (1927). George: *loc. cit.*

is provided.[24] The larger metallographic microscopes are designed so that the auxiliary illuminating apparatus is an integral part of the instrument, and is mounted on an " optical bench " with the microscope and the camera.

METALLOGRAPHIC MICROSCOPES

Since a vertical illuminator can be attached to any microscope, the distinctive characteristic of microscopes designed for the examination of opaque objects is a focusing stage (Fig. 56). Such instruments are commonly called metallographic microscopes, since they are most used in the study of metals. The stage is raised or lowered by means of a rack and pinion, and can accommodate large or small specimens without changing the alignment of the light source with the vertical illuminator. A revolving mechanical stage is desirable.

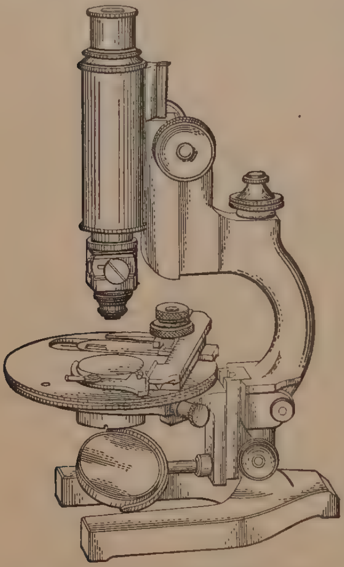

FIG. 56. Metallographic Microscope, with Focusing Rotatable Stage and Mechanical Stage attached. (Spencer Lens Co.)

Works microscopes which may be placed directly upon the surface of large objects (sheet metal, pipe, or non-metallic materials such as paper, enamel, etc.) are manufactured by several firms.[25] That by Leitz (after the design of Stead) rests upon three leveling screws, and is particularly suitable for studies on irregular or curved surfaces.[26] The "Metallographic-Link Microscope" of Leitz possesses a jointed arm which permits the body to be directed at any angle. It is equipped with a hemispherical stage which can be inclined in all directions. The conveniently flexible stand of the Greenough binocular microscope shown in Fig. 25 renders it particularly suitable for examinations of this sort, using inclined illumination.

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[24] A base board, equipped with guides and clamps for the microscope, lamp, and auxiliary condenser, is listed by Leitz.

[25] Stead, Work Shop Microscopes. *Jour. Roy. Micros. Soc.* **1909**, p. 20.

Tassin: Microstructure of Steel Castings, *Ind. Eng. Chem.* **5**, 713 (1913); Metallography as Applied to Inspection, *ibid.* **6**, 95 (1914).

[26] The " capillary microscope " of Bausch & Lomb, if equipped with a vertical illuminator, can be used in a similar manner.

Inverted Metallographic Microscopes are based upon the design of Le Chatelier, whose name they frequently bear. Nearly all[27] large metallographic microscopes for critical work are of this type. By inverting the body-tube, so that the objective is directed upward at a specimen placed over an opening in the stage, the specimen is automatically leveled so that its lower surface is perpendicular to the axis of the microscope. By means of a reflector, the image-forming rays from the objective are turned through 90°, into a horizontal body-tube which carries the eyepiece and is aligned with a photographic camera. An observation tube equipped with an eyepiece, and carrying a reflector at its lower end, may be introduced in the path of the rays, so as to permit viewing the image directly. The illuminating system, with auxiliary condensers, diaphragms and light source, is mounted with the stand and the camera on a substantial horizontal frame. These various essential features are executed according to a number of different designs, the more important and useful of which will be described in some detail.[28]

The Bausch & Lomb Metallographic Microscope shown in Fig. 57 has recently been replaced by a newer model, but it is still in common use, and possesses a widely adaptable illuminating system, which incorporates the features discussed on page 126 in simple and easily understood form.

The instrument consists of an optical bench B 200 cm. long, on short legs. It may rest on a special stand or table or on a board suspended by springs from the ceiling to minimize vibration. This bed B carries sliding stands upon which are mounted the various parts of the apparatus. The radiant placed at the end consists of an arc lamp R; lamps of other types may be interchanged in this mounting. Attached to the lamp housing is a condensing lens C which may be focused by the handle h so as to project an enlarged image of the crater of the arc at c. The character of the light from R may be modified by color filters inserted in the support S; this support may also serve to hold ground glass or a cooling cell. Adjusting screws s, s serve to align the rays from the arc. In front of the condenser C is an iris diaphragm (A, Fig. 55) which serves to control the aperture of the illumination, and may be screened to give oblique or conical illumination. The screen E carries a second condensing lens c, provided with an iris diaphragm (F. Fig. 55) which serves to regulate the field illuminated. This auxiliary condenser projects

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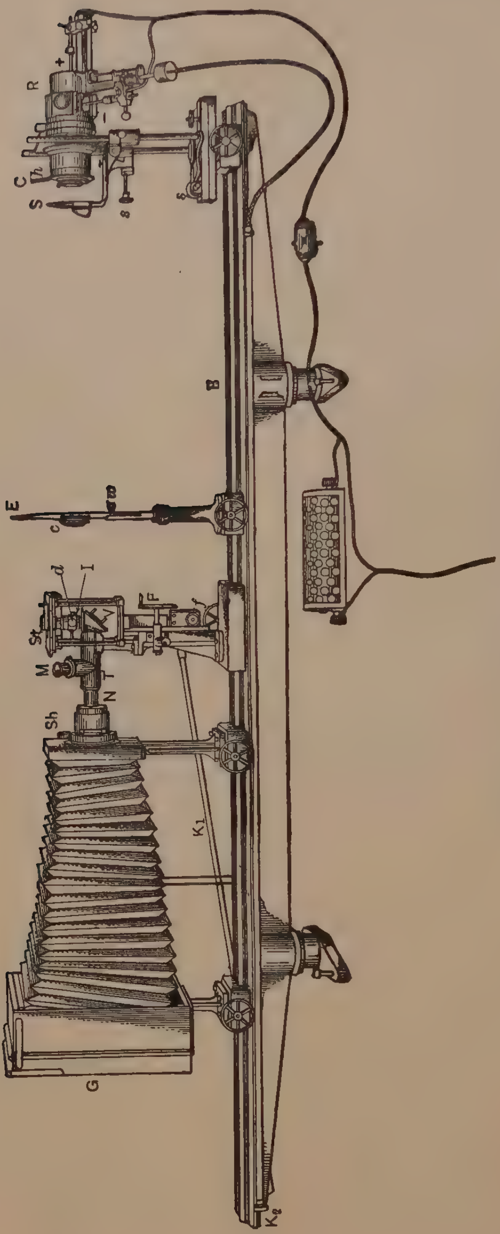
[27] The Martens stand of Zeiss is an exception; it is generally used in a horizontal position.

[28] A number of different types of metallographic microscopes are described by Patterson in Appendix II of Sauveur's *Metallography and Heat Treatment of Iron and Steel* (1926). This article has been reprinted and is obtainable from Bausch & Lomb.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K2

FIG. 57. Metallograph (Bausch & Lomb).

an image of the diaphragm in the opening (A_1 Fig. 55) of the vertical illuminator *I*. By loosening the winged nut *w* this image may be aligned or decentered for oblique illumination.

The compound microscope is attached to the central stand and consists essentially of a stage *St* supported by four pillars attached to the plate *P*, which in turn is movable by worm gear *F* and micrometer screw *f*. The adaptation of a worm gear for raising and lowering the stage ensures that the focus when once adjusted on a specimen will remain sharp even with heavy loads upon the stage, without the use of a special set-screw to lock the focusing mechanism. The microscope proper consists of the tube *T* to which are attached the ocular tube *N* for photography and the observing tube *M*. The objectives screw into adapters which are used with the clutch type of holder (*C*, Fig. 22) and can therefore be very rapidly changed.

The illuminating rays projected by *c* enter the vertical illuminator *I* and are reflected by a disk of plane glass or a half-disk mirror attached to the milled head *d*. The rays from the illuminated object lying polished side down upon the stage pass downward through the disk of the illuminator (or through the unobstructed half when the mirror is employed) and strike a reflecting mirror *V* made of "stellite," from which they are reflected to a reflecting prism mounted at the inner end of *M* whence they are reflected to the eye of the observer. For photography the tube *M* is pulled out a short distance, thus removing its reflecting prism from the tube *T* and allowing an unobstructed passage of the rays through *N* to the ground glass or photographic plate at *G*. Exposures are made by means of the shutter *Sh*.

Since both the coarse adjustment *F* and the fine adjustment *f* are attached to the stage support and not to the tube of the microscope, focusing the instrument cannot disturb the alignment of the radiant. Fine focusing while looking upon the ground glass is accomplished by the Hooke's key K_1 attached to the fine adjustment. The milled head K_2 serves to feed the arc lamp. To prevent dazzling the eyes by the reflection from a highly polished specimen, a cap with black glass is provided to fit over the ocular of tube *M*. There is also furnished with the instrument a cap with a tiny central pinhole which fits over the tube *M*. This device enables the eye to be kept in alignment with the axis of the microscope, when adjusting the illuminating system by looking down the tube at the back aperture of the objective.[29] The optical features of this instrument are typical of other models. Unfortunately, the manufacturers have found that too many users did not take pains to adjust it properly, so a somewhat less flexible illuminating system has been designed to minimize the opportunity for maladjustment.

The present model of the Bausch & Lomb metallograph (Fig. 58) utilizes a vertical illuminator with interchangeable prism and transparent reflectors; the side tube of the illuminator contains a lens (at *l*, Fig. 55) which renders it possible for the objective to form, in the plane of the object, an image of a light source at *F* (which is at a distance less than half the optical tube length). Instead of locating the light source here, its image is projected at this point by the condensing lens C. No adjustable field diaphragm is used (nor the lens c in Fig. 55), the focal lengths of the auxiliary condensing lenses being such that the image of the light source on the specimen is slightly larger than the field of the microscope, with a 5× eyepiece. An iris diaphragm A_1 is attached to the side tube of the illuminator, and functions as an aperture diaphragm. A larger iris diaphragm A in front of the condensing lens C also functions as an aperture diaphragm; it may be decentered by means of a rack-and-pinion r, for oblique illumination; or central stops for conical illumination may be introduced in its aperture. The illuminating system and microscope are permanently aligned on a common base plate B.

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[29] Detailed directions for setting up this instrument and aligning its illuminating system are furnished in pamphlet form by the manufacturers. The description by Patterson (*loc. cit.*) also covers these points.

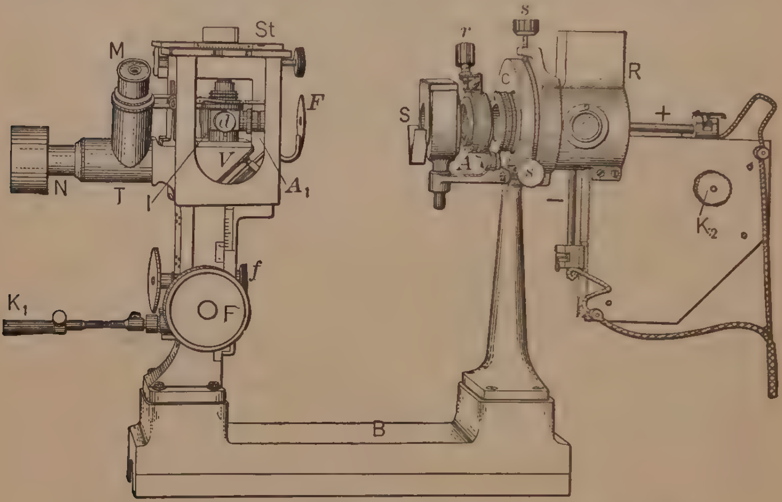

FIG. 58. Microscope and Illuminating System of Metallograph (Bausch & Lomb).

The fine adjustment is designed to carry only the weight of the objective, and the stage is rendered particularly rigid, for photography at high magnifications. The entire instrument is suspended on coil springs with damping pads of sponge rubber, to absorb any extraneous vibration. A special camera back, with counterpoised dark slide, helps to prevent any vibration in exposing the plate after the focus has been carefully adjusted. Instead of the usual ground-glass focusing screen, a viewing tube that swings across in front of the plate may be used for final focusing.

An automatic arc lamp, R, interchangeable with a ribbon-filament, 6-volt incandescent bulb, is used as a light source.

The Leitz "Micro-Metallograph" (Figs. 59 and 60) is provided with an illuminating system which permits excellent control and flexibility of adjustment. The illuminator contains a prism and a transparent reflector, both being mounted on a shaft d, and interchanged by a simple sliding movement along its axis. An auxiliary lens l in a focusing mount is carried in the side-

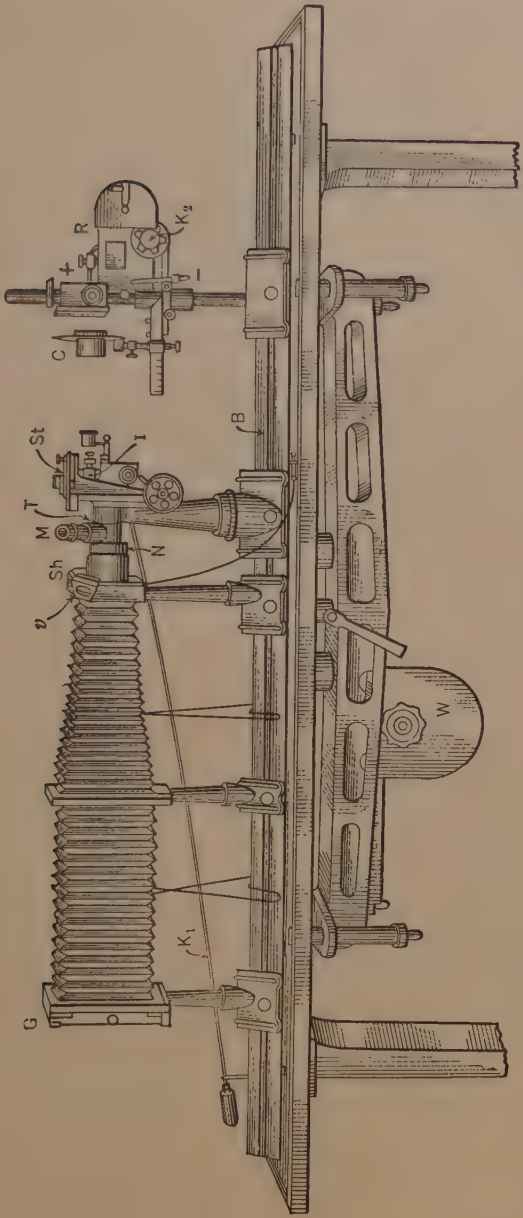
R
+
K₂
−
C
St
T
I
M
N
B
Sh
v
W
K₁
G

FIG. 59. Metallograph (Leitz).

tube of the illuminator, and serves, in conjunction with the objective, to image the iris diaphragm *F* in the plane of the object. This diaphragm controls the size of field illuminated. The aperture *A* of a second auxiliary lens (*c*, Fig. 55) is illuminated by an image of the crater of the arc, projected by the condensing lens *C* of the lamp. The iris diaphragm of this auxiliary lens acts as an aperture diaphragm; it is imaged in the back focal plane of the objective by means of the two auxiliary lenses. The aperture diaphragm is graduated, and is decenterable by a rack-and-pinion *r* for oblique illumination. Conical illumination is obtained by inserting central stops at this point.

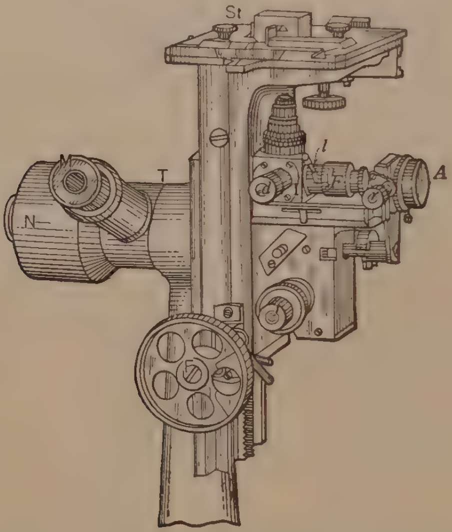

Fig. 60. Microscope of Metallograph (Leitz).

The stand of the Leitz metallograph has a number of valuable features. The optical bench *B* is suspended on springs to minimize vibrations from the floor. A heavy counterpoise *W* balances the suspension irrespective of the position of the parts on the bench, and helps to lower the center of gravity of the apparatus. The automatic arc lamp *R* is mounted so that it may be inclined for illumination of large objects in macrophotography. The microscope is rigidly constructed, with only the weight of objective and illuminator carried by the fine adjustment. The camera is equipped with a viewing aperture at *v*, to aid in centering the specimen for photography. A " focusing extension cabinet " may be attached to the end of the camera, to permit focusing by means of a telescope which swings across in front of the plate.

CHAPTER V

LABORATORY EQUIPMENT

METHODS FOR THE PREPARATION OF MATERIALS FOR MICROSCOPIC STUDY

The variety of problems and materials which come to the chemist for microscopic investigation is so great that the available laboratory equipment should be of wide applicability, and should be selected for possible special needs as well as for the immediate requirements of routine examinations. New lines of work may be developed, necessitating the purchase of additional instruments or the use of different methods of preparing specimens, and the facilities of the laboratory should be flexible enough to provide for such contingencies as they arise.

Laboratory. — The ideal workroom for chemical microscopy should be well lighted by large windows of clear glass. It should be located high enough in the building to be unobstructed by trees or by other buildings, and should face the north if possible. Vibration should be at a minimum and dust should be guarded against. Provision should be made for darkening the room, as an aid in photomicrography and ultramicroscopy, but, if feasible, a separate room should be provided for such special operations; a photographic " dark room " should be connected with it by a " maze." A small hood, and a chemical laboratory work table of standard height, with a sink, should be conveniently accessible, for use in the various operations which are often preliminary to microscopic studies.

Numerous electric outlets are important, for several different light sources are likely to be in continual use. Direct current is almost indispensable if arc lamps are employed, and the wiring should be sufficiently heavy to permit the use of as much as 40 amperes.

Work Tables. — Whatever the design of the work table for chemical microscopy, the following points should be taken into consideration:

1–Comfortable posture of the worker.

2–Favorable lighting.

3–Convenient location of instruments, accessories, and reagents.

4–Ample space for notebooks, and for the operations of preparing specimens.

In general, a rather low table (not over 29 inches high) with cut-out front, as shown in Fig. 61, will be found superior to an ordinary desk. The " cut-out " enables the worker to sit close to the microscope, with both arms resting on the table — a great help in manipulating objects on the stage of the microscope, and an insurance against muscular fatigue. A stool, adjustable in height and with a swivel seat, is indispensable for securing a proper position at the microscope. If the stool is provided with an adjustable back the added comfort thus secured cannot be overestimated.

The indented table top brings the numerous reagents and accessories within easy reach, and has a further advantage in microchemical analyses or investigations in which fumes or corrosive vapors are given off. By placing the microscope at one side of the " cut-out," and the microburner and reagents on the opposite side, these may be kept as far from the instrument as possible. The worker has only to turn to the right or left to change his position from the most convenient one for chemical operations and preparation procedures to that for microscopic observation. The drawers of the table should be designed so as not to restrict such movement.

The table top should be of close texture, and finished in dull black. Coarse-grained woods should be avoided, because of the difficulty of keeping them clean; for this reason the authors prefer table tops of poplar or whitewood, stained black, unpolished and unvarnished and lightly waxed so as to shed water.[1]

A polished or shining top should be avoided, since reflections therefrom are always annoying and very tiresome to the eyes. To guard against injury to the table top, manipulations may be performed over a piece of plate glass; a convenient size is about 12 to 18 inches square.

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[1] A " flat " or " dead black " enamel with carbon black pigment answers admirably and never turns greenish, as aniline black often does.

The table should preferably be placed so as not to face a window, but to be lighted from one side. Such a location will be found less tiring to the eyes than one in which the light comes from directly in front. A table lamp should be provided, to illuminate the notebook and the preparative manipulations; it should be placed low, and carefully shaded so that no stray light will reach the eyes of the worker. The source of artificial light for the microscope may be any one of those described in Chapter III.

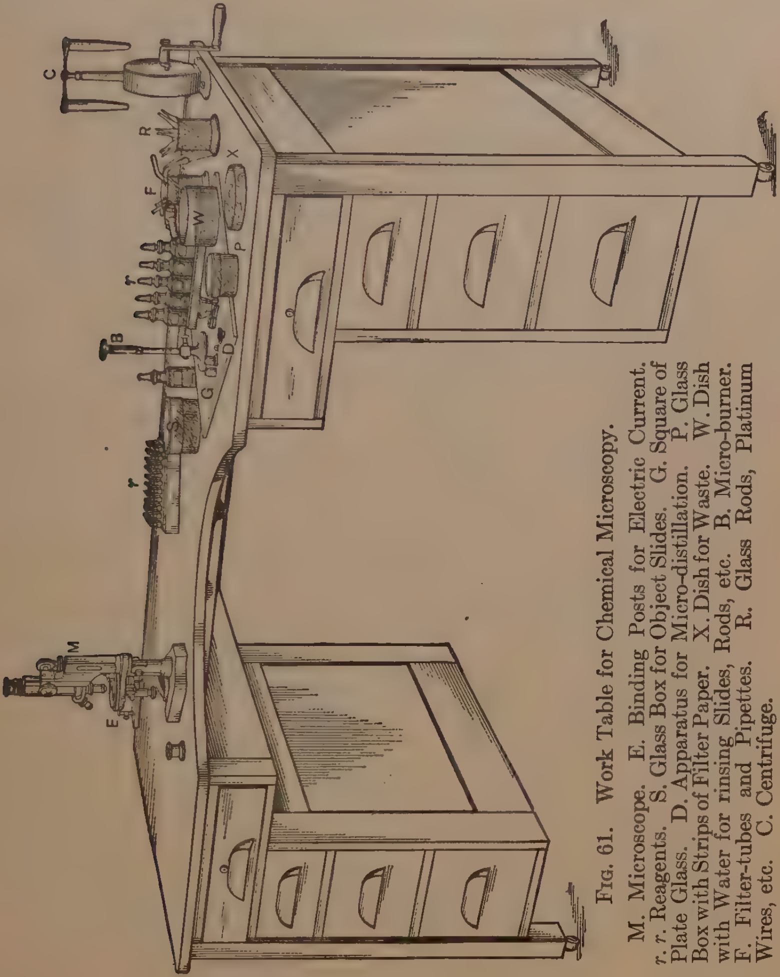

Fig. 61. Work Table for Chemical Microscopy.

M. Microscope. E. Binding Posts for Electric Current. r. r. Reagents. S. Glass Box for Object Slides. G. Square of Plate Glass. D. Apparatus for Micro-distillation. P. Glass Box with Strips of Filter Paper. X. Dish for Waste. W. Dish with Water for rinsing Slides, Rods, etc. B. Micro-burner. F. Filter-tubes and Pipettes. R. Glass Rods, Platinum Wires, etc. C. Centrifuge.

Gas Burners for Microchemical Work. — The microburner shown in Fig. 62 will be found to afford a wide range of usefulness, since it permits heating considerable quantities of material if necessary. It consists of an ordinary Bunsen burner, provided with a small inner tube for a "pilot" flame *F*. This flame is independent of the cock *R*, and is regulated by the small screw *S*, so as to be 3 or 4 mm. high and barely tipped with yellow. If, as often happens, this tiny flame cannot be lowered to the proper size, remove the screw *S*, and drop in the hole a small fragment of soft annealed fine copper wire, replace the screw, and turn until the copper has been crushed sufficiently to obstruct the flow of gas as required. The tube of the "pilot" or "micro" flame should extend flush with the tube of the Bunsen burner. An outer tube *T*, adjustable by the nut *K*, may be raised or lowered as a shield for the tiny flame, when very low temperatures are desired. The height of the support ring *A* may be varied so as to regulate the temperature as when heating liquid in a crucible *C*, or it may be lowered to *B* when not in use. The block of brass *P* serves as a hot plate for heating slides, estimation of melting or subliming points, or evaporation of liquids. It is large enough to possess considerable thermal "lag," and may be maintained at practically constant temperature by adjusting its height above the flame. Substances of known melting points (see Table I, page 210) may be

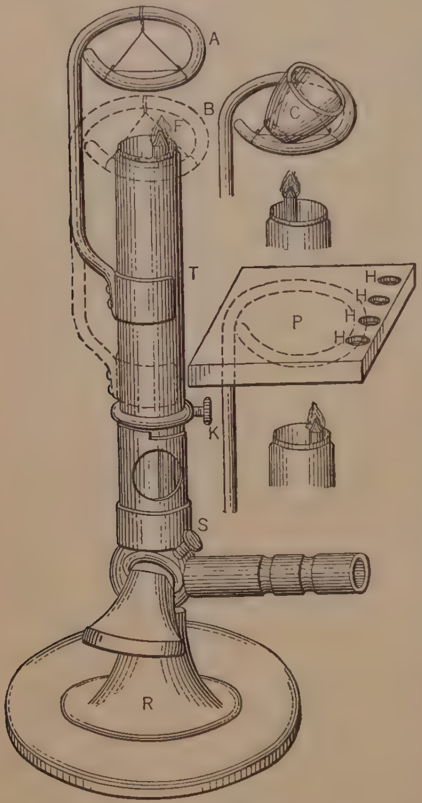

FIG. 62. Micro-burner with Support Ring and Hot Plate.

placed in the holes *H*, *H* to indicate temperatures over any desired range.

For the production of higher temperatures than are possible with the flame of the micro- or Bunsen burner, a small dental blast lamp[2] (Fig. 63) will be found highly satisfactory. Its flame may be regulated to a very fine point, and may be directed downward upon the specimen so that fusion or other effects of heat may be observed under a Greenough binocular microscope.[3]

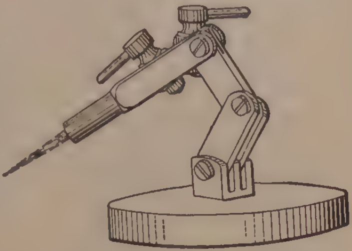
FIG. 63. Grunberg Orthodontic Blow Pipe ×½. (S. S. White Dental Co.).

If compressed air is not supplied to the work table, a mouth blowpipe of the type used by mineralogists will be found convenient. The usual form employed in blowpipe analysis, provided with a platinum tip, should be chosen; if it possesses a hot-blast attachment its usefulness will be greatly increased.

FIG. 64. Forceps with Platinum Tips. (Full size.)

Material to be heated may be held in platinum-tipped forceps (Fig. 64) or may rest on a piece of platinum foil. If fusions are to be carried out, a tiny platinum cup or a loop of platinum wire, such as is used for bead tests in blowpipe analysis, will be found useful.

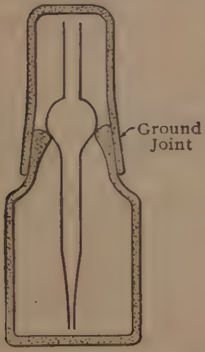

FIG. 65. Dropping Bottle with Pipette and Cap. ×½.

Reagent Containers. — For liquid reagents and mounting media, the bottle shown in Fig. 65 has been found particularly useful, since the material is well protected from all dust or evaporation, and no rubber is used for the dropping pipette. Brown glass is preferable for liquids which may be affected by light.

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[2] Grünberg Orthodontic Blow-Pipe, obtainable from the S. S. White Dental Mfg. Co., Philadelphia, Pa.

[3] Hot stages, for heating microscopic specimens, are described on page 200.

Dry reagents for microchemical analysis are conveniently kept in tiny glass stoppered vials.

PREPARATION OF MATERIAL FOR MICROSCOPICAL STUDY

Although it is always advisable to examine specimens for microscopic study in their original condition, it is only rarely that such examinations can be carried out at high magnifications. Commonly only the gross structure of the objects is evident, and fine details or internal features are practically unobservable. For more than a cursory inspection it is usually necessary to prepare the specimen in an appropriate manner. The actual method employed will depend upon the character of the object and upon the type of study to which it is to be subjected.

In general, it may be said that the procedure employed in preparing the material may either alter or conceal its essential characteristics, or it may reveal them in their true nature. The appearances of most microscopic objects are governed to a large extent by the preparation technique, and its influence must be kept in mind in interpreting the microscopic image in terms of the original structure. Many of the most important advances in microscopical science have been due to improvements in methods of preparation rather than in the manipulation of instruments. In the field of technical microscopy, which covers such an enormous diversity of materials, new procedures must be continually devised, or old ones modified, in order that the improvements in instruments or advances in theoretical knowledge shall not be retarded by the use of inadequately prepared specimens.

Sampling for microscopical study is subject to the same general requirements which apply in ordinary chemical work, with the additional specification that significant structures in the specimen shall be maintained in their original form. This involves some preliminary knowledge of the structure of the material and of the variations which it is likely to exhibit. If the form of the specimen is of interest, the sampling operation must not alter it. If the fineness of powdered material is to be determined, there must be no sorting into coarse and fine fractions. If the material is a mixture of different ingredients, they must be present in the sample in the same proportion as in the original. Unless it is known that the material is highly uniform, samples should be taken from a number of different portions, even if quantitative information is not demanded.

Powdered materials may be sampled and reduced by quartering, or by sifting from a sieve through a "sample splitter." If aggregates are present, they should be picked out and examined separately, as well as being broken up and mixed with the rest of the material. It may be desirable in some cases to suspend the powder in liquid, and to take an aliquot part of the suspension, in order to insure perfect mixing and uniformity.[4]

Massive materials, such as alloys, should have samples taken from various points of their cross-section and longitudinal section, unless it is possible to include the entire section in the preparation which is to be studied. Textiles should be examined with respect to each type of warp and woof thread present. Sheets of paper should be folded and small pieces cut from various parts.

If quantitative information is desired, the results from various samples should be compared; if agreement is poor, larger samples, or more determinations on each sample, should be made. Since few materials are very uniform under the microscope, samples involving hundreds of individual particles, grains, or fibers may need to be studied.

Separation and Concentration by Manual Operations. — If the purpose of sampling is to obtain a small amount of material for detailed study, it is often important to be able to secure this sample without contamination by adjacent substances, and to know exactly where the sample was taken. For such work a Greenough binocular microscope or a simple magnifier is invaluable, since it enables powdered materials to be sorted, coatings or incrustations to be separated from underlying material, and single grains in aggregates to be dug out, all under microscopical observation. The material thus collected may be further examined at higher magnifications, tested with polarized light, or analyzed microchemically.[5]

Manual separation of the different constituents of a sample, though sometimes laborious, has the distinct advantage that a minimum of mechanical or chemical treatment is applied, and the separated portions are unaffected by the process. It is, of course, only applicable to materials which are recognizably different, even at low magnifications, and which are not too minute to be handled.[6]

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[4] Weigel: Size and Character of Grains of Non-Metallic Mineral Fillers. *Bur. Mines. Techn. Paper* **296**, p. 7.

[5] Johannsen: *Manual of Petrographic Methods* (1918), p. 557.

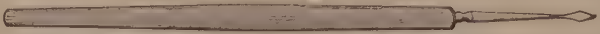

Fig. 66. Spear Point Dissecting Needle. ×⅔.

For the sorting or dissection of microscopic objects small instruments such as are used in histology are very useful. Needles (Figs. 66, 67), knives, forceps, scissors, and similar tools are suitable for soft materials. Forceps should have very fine jaws, only slightly corrugated and meeting so accurately that tiny particles may easily be picked up; the tips may need to be reshaped by the user (Fig. 68). A glass rod drawn to a fine point, or a platinum wire sealed to a piece of glass tube, are very convenient for picking up small grains of material (Fig. 69); they may be moistened by breathing upon them, or rendered slightly sticky by a trace of Canada balsam or vaseline. A miniature spatula, hammered from heavy platinum wire, is useful for handling particles of all sizes, or for scraping up loose material (Fig. 70). Bits of glass rod drawn to hair-like fineness and cut

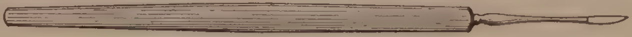

Fig. 67. Knife Needle. ×⅔.

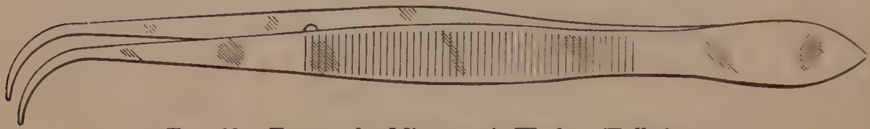

Fig. 68. Forceps for Microscopic Work. (Full size.)

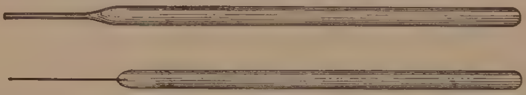

Fig. 69. Drawn-out Glass Rod and Platinum Wire for handling Reagents.

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[6] It should be borne in mind that the microscope in effect serves to separate materials, for by spreading the mixture in a thin layer particles of the different ingredients may be observed entirely isolated from other constituents. These may be tested *in situ* optically, chemically, and by their melting points, and frequently can be identified without actually being removed from the mixture.

into lengths of about 50 mm. will be found useful in dealing with very minute particles, or applying exceedingly small drops of reagents. They may be thrown away after using. For collecting fragments of material which are covered by liquid, a plentiful supply of capillary pipettes, made by drawing out-glass tubing as shown in Fig. 71, should be at hand; these may be equipped with rubber bulbs if desired, and should be discarded after use.

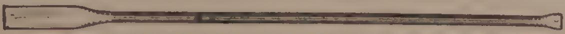
Fig. 70. Platinum Spatula. (Full size.)

Aggregates of hard material, such as refractories, minerals, or alloys, may require considerable force to separate certain constituents, and for this the smaller sizes of chisels and excavators used by dentists are excellent. They are made of good steel, and may be obtained in a variety of shapes, or re-shaped by grinding. Steel sewing needles of large size or knitting needles, may be heated, shaped and hardened, or shaped by grinding to make special tools. For cutting or chiseling the hardest materials a writing diamond is invaluable.[7]

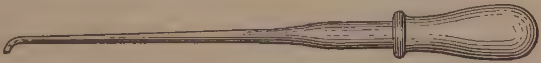
Fig. 71. Capillary Pipette.

The smallest size jeweler's vises and tool-maker's clamps are very convenient for holding material which is being cut or chiseled under the microscope (Fig. 24) since they may be fastened to the stage itself. If the sample is inconvenient to hold it may be partially embedded in sealing wax, in a low-melting alloy, or in plaster-of-paris or other cement.

For pulverizing aggregates of material, preparatory to further separation, a small agate mortar may be used (Fig. 72). A "diamond" mortar is preferable for hard and brittle substances, since it prevents any loss of the fragments. An unglazed porcelain "streak plate," such as is used by mineralogists, affords a simple means of obtaining a small quantity of the material in powder form; a piece of ground glass may be used,[8] or, better, an object slide of fused silica with ground surface.

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[7] A modified form of writing diamond has been devised by Putnam, Roberts, and Selchow: *Amer. Jour. Science.* **15**, 99 (1928) and used by them in sampling mineral grains for microscopic chemical analysis. This tool is made by the Arthur Crafts Co., Diamond Cutters, Boston.

For accurately controlled sampling, the micro-drill described by Fairbanks: *U. S. Bur. Mines. Repts. of Invest.* **2613** (June, 1924) may be employed to advantage. It is centered on the specimen by means of the microscope, and the depth of the depression made can be controlled.

In separating coarsely powdered materials it is advisible to spread them over a flat surface such as the glass stage of the Greenough binocular microscope, or a piece of plate glass; a light or dark colored background may be placed beneath the glass, to give better contrast. The different kinds of grains may be picked up and placed in separate watch-glasses, or in the hollows of a porcelain "depression plate." If very fine particles are to be dealt with, it is more convenient to push them together into heaps, rather than to attempt to pick them up.

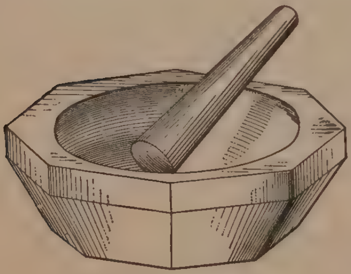
FIG. 72. Agate Mortar. (Full size.)

Although it is possible, by supporting the hand firmly on a rest, to handle and separate fairly small grains under the microscope, such procedure is not adequate for the manipulation of very tiny particles, or the dissection of fine structures.

Various methods have been employed to substitute mechanical for muscular movement,[9] and recently two types of **micro-manipulators**, designed for work at high magnifications, have been placed on the market. Chamber's apparatus[10] (manufactured by Leitz) consists of supports for heavy arms to which the needles or pipettes used are attached. These arms are hinged by springs which are flexed by screws working against them, and thus a delicately controlled movement is imparted to the point of the instrument used. A separate spring arm and support is necessary for each needle or pipette in use. The Peterfi micro-manipulator[11] (manufactured by Zeiss) is a more flexible apparatus, in that it has rack-and-pinion coarse adjustments, and a wider range of movement.

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[8] The use of "streaks" as samples for microchemical testing is discussed by Gaubert: *Comptes rendus*, **177**, 960 (1923).

[9] Barber: *Philippine Jour. Sci.* **9B**, 307 (1914).
Blake: *Amer. Jour. Sci.* **37**, 535 (1918).
Hurst: *Trans. Amer. Micros. Soc.* **44**, 224 (1926).
Taylor: *Univ. California Pub., Zoölogy* **26**, 443 (1925).

[10] *Proc. Soc. Expt. Biol. Med.* **19**, 85 (1921).
Anat. Record. **24**, No. 1 (1922).
Jour. Roy. Micros. Soc. **1922**, 373.
Jour. Bacteriol. **8**, 1 (1923).
Lee: *Microtomist's Vade Mecum.* (P. Blakiston's Son, Philadelphia, 1928), Chapter XXX.
McClung: *Handbook of Microscopical Technique* (P. B. Hober, New York (1929), pp. 39–73.

The tiny instruments which are controlled by these micro-manipulators are usually made by drawing out glass rod or tube to fine points, even as small as 1 μ in diameter. Forceps, needles, pipettes, heating points, magnets, or electrodes may be operated by the mechanical movements, under magnifications as high as 1000×. Only very slight force can be applied by such delicate tools, but somewhat stronger and coarser ones are suitable for most chemical work, and are of great value in separating specks and foreign matter from various materials, as a preliminary to their identification by microchemical means. The micro-manipulator also has possibilities as a method of applying a tiny drop of reagent to a single grain of mineral or metallic substance, as a substitute for separating the grain from its surroundings before testing it chemically.

Separation and Concentration by Mechanical Operations. — If the properties of the ingredients of the specimen permit, some sort of mechanical separation is preferable to sorting by hand, and may be necessary in case the constituent particles are not strikingly different at low powers. The operations used may be no more complicated than shaking the pulverized sample on a flat surface (analogous to a jig table) or winnowing in a current of air. In many cases screening of a powdered sample will effect a separation into coarse and fine fractions, in each of which certain ingredients predominate.[12] This is particularly true in the case of mixtures which have been made up from materials of varying finenesses, as in the case of stock feeds.[13]

Magnetic separation is applicable to many minerals which are only feebly magnetic in mass.[14] A bar or horseshoe magnet is sufficient for ordinary work, where distinctions need not be based on the quantitative degree of magnetic susceptibility. Fairbanks[15] uses dielectric separation methods as a means of obtaining fine particles of a single ingredient for microchemical analysis.

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[11] *Zeits. wiss. Mikros.* **41**, 263 (1924).

Mikrurgische Methodik, in Aberhalden's *Handbuch der biologischen Arbeitsmethoden*, Abt. V, Teil 2, Lief. 124 (Urban & Schwarzenberg, Berlin).

Krause: *Enzyclopädie der mikroskopischen Technik.* Vol. II (1926), pp. 1424–35.

[12] Tiny sieves may be made from silk bolting cloth of suitable "mesh" stretched on a frame, and discarded after use.

[13] Wallis: *Analytical Microscopy* (E. Arnold, London, 1923), p. 14.

[14] Johannsen: *op. cit.*, p. 538.

The specific gravities of the ingredients of a mixture may be used as a basis of their separation and concentration. If a liquid of the proper density is chosen, certain of the constituents will sink and others will float in it.[16] By proper control, the parting may be made quite definite, and mixtures of several ingredients may be fractionated into their different components almost quantitatively. Constituents of ores, foodstuffs, drugs, and other heterogeneous materials may be separated in this manner. It is necessary that the grains of material should not be aggregated, and that they should be small enough to consist of one substance only. Most important of all, the liquids used should have no chemical action on the sample. Minerals and other insoluble materials may be separated by Thoulet's solution of potassium mercuric iodide ($G = 3.2 -$), Klein's solution of cadmium borotungstate ($G = 3.2 -$), or similar aqueous solutions of inorganic compounds, but for general work organic liquids are preferable. Bromoform ($G = 2.6$), acetylene tetrabromide ($G = 2.95$), and methylene iodide ($G = 3.3$), are the most useful of these. By mixing with liquids of lower density, such as benzene, chloroform, or carbon tetrachloride, any desired value may be obtained.[17] For small amounts of material a separatory funnel or a sedimentation glass is satisfactory. The collected grains may be washed free of adhering liquid and dried.

Finely divided material temporarily suspended in water or other liquid may be concentrated by a number of methods, of which settling is the simplest, since the concentrate is easily handled. The apparatus illustrated in Fig. 73, commonly known as Spaeth's sedimentation glass, is very useful for this purpose. The suspension is allowed to settle, the stopcock being open upward, as shown. After subsidence has taken place gentle stirring will dislodge any material clinging to the side of the vessel and it will fall to the bottom. The stopcock is turned a quarter turn and the liquid emptied out. The stopcock can then be removed, with the sediment contained in the conical depression and with but little of the supernatant liquid. Fractional sedimentation may be possible, if suspended particles having different rates of subsidence are dealt with.[18]

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[15] *Laboratory Investigation of Ores* (1928), Chap. V.

[16] Johannsen. *op. cit.*, pp. 518–557.

Wallis: *op. cit.*, p. 48.

[17] An exhaustive study of the most useful and economical liquids for specific gravity separations is given by Sullivan: *Bur. Mines. Technol. Paper* **381** (1927).

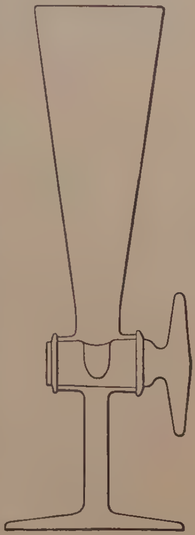

Fig. 73. Spaeth Sedimentation Glass.

If settling is slow, on account of the fineness of the particles or for other reasons, centrifugal force may be employed to accelerate it. The usual conical tubes (15 cc.) are useful for samples of moderate size, while for smaller quantities of liquid (less than 1 cc.) the "haematocrit" attachment used for centrifuging blood serum may be employed, with higher speeds if necessary. If a small amount of solid is suspended in a large bulk of liquid, a larger tube and heavier centrifuge are required. The most convenient form of apparatus for this purpose consists of a separatory funnel fitted with a stopcock of the type provided in the Spaeth sedimentation glass (Fig. 74). This simplifies greatly the collection of the solid material after it has separated. Ordinary centrifuge tubes allow the solid to collect in the tip, from which it must be removed by a capillary pipette, preferably after the supernatant liquid has been pipetted off. It will be found more convenient to employ tubes drawn down to a fairly long pointed end, which may be cut off by a file scratch just above the sediment, thus permitting easy access to the solids. When properly drawn down, tubes of this form can be used several times by simply sealing the end; the tubes are centered and held in the centrifuge by perforated corks. The

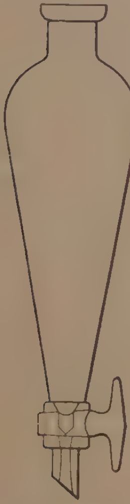

Fig. 74. Sedimentation Funnel for Large Centrifuge.

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[18] Wallis: *op. cit.*, pp. 28, 50.

[19] *Jour. Amer. Chem. Soc.* **27**, 104 (1905).

tubes devised by Richards[19] for the separation of small quantities of crystals from their mother liquor are useful for similar purposes. Their manipulation is evident from Fig. 75.

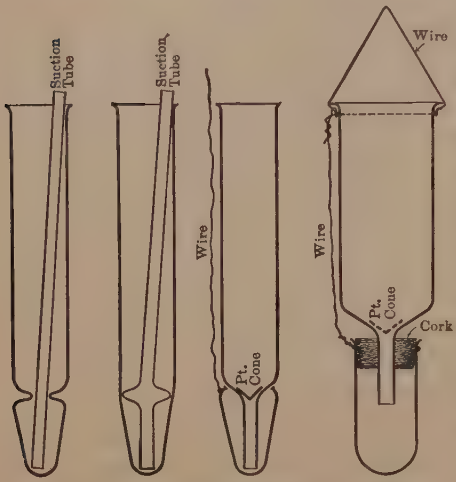

FIG. 75. Richards Tubes for Centrifugal Separations.

The collection of fine suspended particles which cause turbidity in oils, lacquers, and other liquid or solid materials may be accomplished most conveniently by means of the centrifuge. The sample may be diluted or dissolved by an appropriate liquid, to render the separation rapid and complete.

Filtration as a means of collecting suspended solids for microscopic study presents no unusual problems. However, if filter paper is used it may be difficult to collect the solids or to free them from stray fibers. (The filtration of small quantities of liquid is discussed in Vol. II.) The Sedgwick-Rafter method[20] eliminates the use of paper, sand being used as a filtering medium. After the suspended matter from a quantity of liquid has been collected on the bed of sand in the apex of the funnel, sand and sediment are removed, and separated from each other by washing and decantation; the sand is heavy and coarse and settles out quickly, while the concentrated suspension of sediment may be collected and examined.

For certain purposes it may be simplest to dissolve or otherwise to remove all but one of the ingredients of a mixture, in order that it may be more clearly visible. Such procedures are matters of chemistry, and will depend on the nature of the sample. Similarly, it may be desirable to ash the specimen, in order to study

[20] *Standard Methods of Water Analysis* (1925).

Whipple: *Microscopy of Drinking Water* (John Wiley & Sons, New York, 1927), p. 90.

the structure and distribution of its inorganic constituents *in situ.*[21]

Maintainence of the original structure of the specimen is frequently important if the relationship of the different constituents is to be investigated microscopically. The composition of the sample is revealed more clearly, and the history and development of its structural elements are much more plainly evident if their arrangement has not been disturbed by any method of preparation such as crushing or tearing apart with dissecting instruments. The progress of deteriorating influences such as corrosion, solution, or other chemical attack is readily followed under such circumstances, and the effect of various reagents on the material may be observed microscopically in a very definite manner.

The ideal method of preparation of a specimen for such studies would be to pass a mathematical plane through it, and to examine the structures which this surface interests. In the case of transparent material, a very thin slice of the specimen would represent the internal features intersected by it. The procedures for approximating such ideal preparations vary greatly with the physical character of the sample; for soft materials the methods of plant or animal histology may be adapted, whereas for hard specimens the technique of metallography or petrography is applicable.

Surfacing or sectioning soft materials which can be cut by a knife is a very easy matter if the specimen is reasonably firm and tough. Transparent material may be cut in thin sections for study by transmitted light, but for relatively opaque materials such as coats of paint[22] the examination of a smoothly cut surface by reflected light is usually sufficient. Reasonably accurate sections or surfaces can be prepared by freehand cutting, using a *very* sharp knife (such as a " pattern " or wood carver's knife) or a strong razor. A safety razor blade in a suitable holder furnishes an excellent edge, and can be discarded if injured by hard or gritty material. The specimen may be held in the fingers, or between two pieces of pith, cork, or other soft material. To avoid risk of tearing or distorting the structure, it is best to remove only a very thin slice at a time, even if the *remaining* surface is to be studied. A drawing cut is permissible, but care should be taken not to compress or otherwise deform the object.[23]

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[21] Molisch: *Mikrochemie der Pflanze* (Jena, 1921), pp. 9–11.

Schoeller: *Berichte* **55**, 2191 (1922).

Policard: *Comptes rendus* **176**, 1012, 1187 (1923); *Bull. soc. chim.* **33**, 1551 (1923).

Pregl: *Mikrochemie* **2**, 75 (1924).

[22] Maxwell: *Chem. Met. Eng.* **28**, 850; **29**, 964 (1923).

If more accurate control of the cutting operation is desired, a **microtome** is essential. A microtome consists of a holder for the specimen, a feed mechanism for regulating the thickness of the cut, and a guide for the knife. Unless serial sections are required, the simpler types of microtomes are preferable for industrial use, since their construction is rugged and not likely to be strained by the force necessary in cutting tough objects.

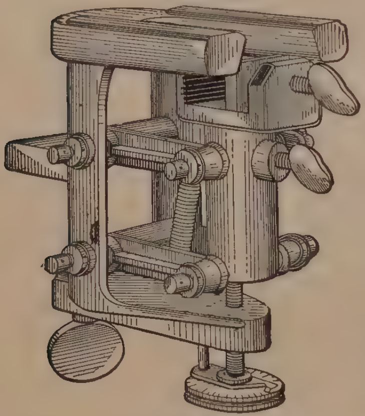
FIG. 76. Small "Table" Microtome. (Spencer Lens Co.)

A sturdy microtome of simple construction (Fig. 76) which can be clamped to the table top answers admirably for most work.[24, 25] The jaws for clamping the specimen will accommodate as large pieces as it is feasible to cut. The knife is held in the hand and is guided by the flat strips of glass on the top of the instrument. Cutting may be carrried out with any keen-edged knife, but a "section razor" is more easily sharpened to a smooth, sharp edge. A "botany" razor having a heavy blade is satisfactory; the steel should be hard enough to hold an edge but not so brittle that it is chipped by hard particles which may be encountered in the specimen.[26] For very tough material, a heavy chisel-like knife is necessary.

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[23] Carpenter: *The Mircoscope and Its Revelations* (London, 1901), p. 458.

[24] If a more elaborate microtome is desired for cutting very thin sections, the new precision sliding microtome manufactured by Spencer Lens Co. is preferable to instruments of the rotary type, since its mechanism is much stronger. A large microtome knife is better than a razor, on account of the stiffness of its blade. Holders may be obtained to permit safety razor blades to be used in microtomes of this type, so that the labor of sharpening blades, which it may have been necessary to misuse on hard and gritty material, is largely eliminated.

[25] An improvised microtome is described by Speare and Moore: *Ind. Eng. Chem.* **17**, 894 (1925).

Microtomes are used almost entirely for cutting thin sections of material, but Lucas[27] and others have recently been successful in surfacing alloys by means of a substantial and rigid microtome.

It is frequently desirable to soften the specimen by soaking in water, or by other treatment[28] before attempting to section it by means of a knife. Small pieces of material which are sufficiently strong and rigid may conveniently be held between pieces of elder pith and clamped in the jaws of the microtome; a few drops of alcohol applied to the pith cause it to swell and to support the specimen firmly in place.

If the material to be sectioned is friable or of poor tenacity, if it consists of structures loosely bonded together or of varying toughness, or if very thin sections are required, some sort of **embedding** procedure must be applied.[29]

The aim of all such methods is to permeate the material thoroughly with a substance which will be firm enough to maintain every part of the structure in its original relative position, and which may be sectioned readily by the microtome. Obviously, the embedding operation must not alter the structure or chemical constituents of the specimen, and the choice of a method will be governed largely by the nature of the substance and its susceptibility to heat or solvents.

Most dry porous materials may be infiltrated by molten paraffin or harder vegetable waxes, provided they are not affected by a temperature of 60–70°, and contain no oil-soluble ingredients. Paper,[30] textile fibers,[31] leather, and similar substances with continuous air voids may be embedded very rapidly by this method. The block of wax containing the specimen is trimmed to a small size and fastened on a small piece of wood by hot wax, to give a firm base for the jaws of the microtome. Even if penetration is imperfect, the wax surrounding the exterior of the specimen may support it firmly enough for sectioning.[32]

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[26] Gage: *The Microscope* (1925). Microtomes and Section Knives, pp. 370–5.

Kingsbury and Johannsen: *Histological Technique*, p. 23 (John Wiley & Sons, New York, 1927).

Krause: *Enzyclopädie der mikroskopischen Technik*, Vol. II (1926), pp. 1528–48.

[27] Microtome Methods for the Preparation of Soft Metals for Microscopic Examination. Pamphlet 1654-E. *Mining and Metallurgy*, Feb. 1927.

[28] Kisser: *Zeits. wiss. Mikros.* **43**, 346 (1926); **43**, 495 (1927), recommends playing a steam jet on the spot to be cut, to soften and moisten materials such as wood and horn.

[29] Lee: *op. cit.*, Chap. VII.

[30] Tingle: *Paper Ind.* **9**, 418 (1927).

[31] Herzog: *Mikroskopische Untersuchung der Seide und Kunstseide* (J. Springer, Berlin, 1924), p. 21.

See also Johnson: *Amer. Dyestuff Rept.* **18**, 37 (1929); Garner: *Ind. Chemist*, **5**, 147 (1929).

A syrupy solution of collodion may be used as an infiltrating material for dry, fibrous material; on drying, it shrinks somewhat, but this is not objectionable in the case of bundles of textile fibers.[33] If desired, the collodion may be hardened by immersing in chloroform instead of by evaporation. The specimen, embedded in collodion of a tough, almost horny texture, is preferably surrounded by paraffin as a support for sectioning.[34] Sections of either the paraffin or the collodion methods may be mounted directly in Canada balsam.

If it is necessary to avoid the use of organic solvents, an aqueous embedding medium may be employed; this is also particularly applicable to moist objects. A concentrated solution of gelatine, or gelatine and glycerine, is kept warm and allowed to soak into the specimen. It sets when cold, and may be hardened by formaldehyde and alcohol.[35]

Another procedure, which involves a minimum of chemical treatment, is to freeze the wet specimen by means of an ether spray or a carbon dioxide expansion chamber attached to the microtome. The freezing is continued during the sectioning operation. In order to avoid the growth of large ice crystals which would render the cutting difficult and the section brittle, gum arabic, gelatine, sugar, or other substances may be added to give a tougher and finer grained mass.[36] Sections prepared by the gelatine or the freezing method may be mounted in glycerine or in glycerine jelly.[37]

If very thin sections of delicate material are to be cut, the above rapid methods may be inadequate, and more elaborate technique may be necessary. Either paraffin or collodion may be used as an impregnating medium, but a number of stages are required to dehydrate the material completely, to render it permeable to the infiltrating material, and to remove this material without altering the structure of the specimen.

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[32] Rubber, isolated coats of paint, celluloid, and other non-porous materials may be conveniently supported in this manner.

[33] Willows and Alexander: *Jour. Textile Inst.* **12**, 199 (1921).

Lawrie: *Textile Microscopy* (E. Benn, London, 1928), p. 131.

[34] Herzog: *loc. cit.* p. 19, simplifies the procedure in the case of textile fibers by making a single freehand cut and examining the exposed ends of the fibers by reflected light.

[35] Lee: *op. cit.*, p. 95.

Clegg and Harland: *Jour. Textile Inst.* **14**, T489 (1923).

Lawrie: *op. cit.*, p. 129.

[36] Guyer: *Animal Micrology* (Univ. Chicago Press, 1917), Chap. VIII.

Johannsen and Kingsbury: *Histological Technique* (1927), p. 35.

McLaughlin and O'Flaherty: *Jour. Amer. Leather Chem. Ass.*, **22**, 323 (1927).

[37] Freezing has also been used to harden rubber sufficiently for cutting without yielding beneath the knife, by Depew and Ruby: *Ind. Eng. Chem.* **12**, 1156 (1920). Superficial vulcanization with sulphuryl chloride is also effective for this purpose. Green: *ibid.* **13**, 1130 (1921); Speare and Moore: *ibid.* **17**, 936 (1925).

The **paraffin method**[38] involves a preliminary gradual dehydration of the specimen by soaking successively in a series of aqueous alcohols, of increasing concentrations, until absolute alcohol is reached. The alcohol is replaced by soaking in a liquid which is miscible with paraffin, such as xylene or cedar oil.[39] The specimen is then soaked in molten paraffin, which is changed several times, until it is thoroughly impregnated. The wax is then chilled and mounted on a block; sections are cut, and fixed to a microscope slide. The paraffin is extracted by xylene or benzene, and this is replaced by Canada balsam or other resinous mounting medium. If, however, it is necessary to stain the section with an aqueous stain, it must be passed from the xylene through the series of alcohols until it is hydrated, and after staining it must be dehydrated as at first, before mounting in balsam.

The **collodion or "celloidin" method**[40] necessitates a preliminary dehydration through the series of alcohols, as in the paraffin method. From 95 per cent alcohol the specimen is transferred to a mixture of alcohol and ether, both preferably anhydrous. It is next soaked successively in collodion solutions of increasing concentrations, the last being a viscous syrup. The sample, surrounded by semi-gelatinous collodion, is mounted on a block of wood by means of more collodion, and allowed to stiffen somewhat by evaporation. It is then placed in chloroform, which hardens the collodion and renders it tough and almost horny. The specimen is finally transferred to aqueous alcohol or a mixture of castor oil and xylene, in which solutions it may be stored indefinitely. For sectioning, the specimen is clamped in the microtome and cut with a drawing stroke, the knife being kept lubricated with the liquid in which the specimen was stored. After the section has been attached to a microscope slide, the collodion may be removed by a solvent, but it is often left in place as a support for the fine structures of the specimen. Dehydration may be necessary before mounting, if the mounting medium is not miscible with that in which the specimen was stored. Castor oil or phenol in xylene give transparent preparations which may be examined directly or mounted in balsam.

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[38] For detailed directions covering all stages of this method see
McClung: *Microscopical Technique* (P. B. Hober, New York, 1929), pp. 1–38, 108–164.
Lee: *Microtomist's Vade Mecum* (P. Blakiston's Son, Philadelphia, 1928), Chap. VIII.
Kingsbury and Johannsen: *Histological Technique* (John Wiley & Sons, New York, 1927), pp. 17–26.
Gage: *The Microscope* (Comstock Pub. Co., Ithaca, N. Y., 1925), pp. 375–86.
Carpenter: *The Microscope and Its Revelations* (London, 1901), Chap. VII.
Wilson and Daub: *Jour. Amer. Leather Chem. Ass.* **21**, 193 (1926).
Turley: *idem.*, p. 117.
McLaughlin and O'Flaherty: *idem.*, p. 338.
Wilson: *Chemistry of Leather Manufacture* (Chemical Catalog Co., New York, 1923), pp. 15–20.
Herzog: *Mikroskopische Untersuchung der Seide und Kunstseide* (J. Springer, Berlin, 1924), p. 21.

[39] So-called "clearing" agents.

[40] For detailed directions covering all stages of this method, see
McClung: *loc. cit.*
Lee: *op. cit.*, Chap. IX.
Kingsbury and Johannsen: *op. cit.*, pp. 26–32.
Gage: *op. cit.*, pp. 387–92.
Carpenter: *loc. cit.*
Wilson and Daub: *loc. cit.*
Lawrie: *Textile Microscopy* (E. Benn, London, 1928), p. 123.

Both of the above methods of embedding are very time-consuming on account of the hours or days required for the penetration of each liquid. It is this slowness which minimizes the risk of delicate tissues being deformed by osmotic forces due to too sudden changes in the surrounding fluid. The paraffin method is rather more rapid than the collodion method, and thinner sections may be cut, but the sample is not so firmly held together.

Sections should ordinarily be cut sufficiently thin to permit structural elements to be seen in single layers. Cross-sections of fibers or coatings should be no thicker than the details which are to be studied, if spurious images are to be avoided. The collodion method is satisfactory for sections as thin as 10 μ, but for thinner sections, down to 1 μ, the paraffin method must be used. Thin sections can be cut only with a rigid, well sharpened knife and an accurately working microtome. They must be handled with great care, in order to avoid breaking the delicate slice of the specimen contained in the embedding material, and after this supporting matrix is removed they must not be subjected to any mechanical force. For this reason the sections are usually fixed to a microscope slide by an adhesive before any attempt is made to remove the paraffin or collodion, or to cover them with a mounting medium and cover-glass.

Serial sections (cut successively) are useful in tracing a given structure through a thickness greater than that of a single section, and may be used to construct three-dimensional models.[41] Sections in two or more coordinate planes are frequently of value in interpreting the longitudinal and tranverse structure of an object.

Surfacing or sectioning hard materials which cannot be cut by a knife in their original condition, or rendered soft enough by special treatment, depend principally on grinding methods. Certain brittle materials which break cleanly may give a fractured surface which is suitable for study by reflected light; porcelains, glazes, and vitreous enamels may be thus examined for coarse structural features. In general, however, the surface must be prepared by abrasion, the methods and materials used depending on the hardness and other properties of the specimen. The surfacing of metals will be discussed in detail, as a typical procedure on which other surfacing operations may be based.

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[41] Gage: *op. cit.*, pp. 399–423.

Krause: *Enzyclopädie der mikroskopischen Technik.* Vol. III (1927), pp. 1924–2020.

Surfacing of metals for microscopic study must be carried out with as little superficial distortion as possible. Every one of the steps of the procedure described below is carried out so as to minimize any surface flow of metal, and to avoid any burnishing effect. The removal of material to give a smooth surface is accomplished by *abrasive* action, which is progressively more delicate as the succeeding stages render the specimen more suited for examination at high powers. "Polishing," in the sense of producing a mirror surface by any means, is less desirable for microscopic preparations than is cutting by very fine abrasive particles which leave the surface covered with minute furrows but which do not obscure its inner structure by a film of flowed metal.[42] Considerable practice is necessary to develop the firm but light touch which is essential to the success of the various surfacing operations.

The sample may be reduced to a convenient size and shape (approximately 1 cm. in each dimension) by means of a hack saw, if it is not too hard to be cut. Hardened steels, white cast iron, and similar materials must be cut by a rapidly rotating abrasive disk. In order to avoid undue heating, the specimen should be kept wet or the disk should run in water. The material in the immediate vicinity of the cut surface is always deformed or otherwise altered by the cutting operation, and must be removed if the true internal structure is to be laid bare. At the same time the surface must be rendered plane.

As a preliminary to the roughing operation, the edges of the sample are usually beveled to facilitate the later stages of surfacing, and to permit the fingers to grip it strongly without pain. In the case of very hard materials the surface to be prepared is then held lightly against the side of an abrasive wheel. The sample must be gripped tightly in the fingers, however, and must not be allowed to turn so as to produce a rounded or faceted surface. Movement back and forth radially across the wheel will help to wear down its surface evenly, and to prevent glazing. The sample must not be allowed to become warm if its structure is likely to be altered by heat; the face which is being ground may be very much hotter than the body of the piece which is touched by the fingers, so the safest procedure is to keep the wheel wet and to cool the metal by frequent immersion in water. A properly ground specimen should show a plane surface, with all striations parallel and approximately equal in depth; more than sufficient material to remove the marks of the saw should be ground off.

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[42] The theory of polishing is discussed by Rayleigh: *Proc. Roy. Inst.* **16**, 563 (1901); see also French: *Trans. Faraday Soc.* **18**, 42 (1922).

Beilby: *Aggregation and Flow of Solids* (London, 1921), emphasizes the practical impossibility of preventing a very slight amount of surface flowage.

The abrasive wheel used should be of fairly hard grade, so as not to wear away too rapidly and lose its plane surface. A medium fine grain is desirable for most work, 80–P "Alundum" being satisfactory. As a second stage in grinding, a finer grained wheel, such as 200–M "Alundum," is useful. Other grades and grains of abrasive wheels may be preferable, if much work is to be done on very soft or very hard metals. The wheels should run at about 1200 r.p.m. and should be mounted truly and dressed when necessary.

Wherever possible, it is preferable to avoid the use of abrasive wheels for roughing, since it is difficult to obtain perfectly plane surfaces, and the risk of heating or " flowing " the surface of the metal is considerable.

Filing by hand is recommended for all metals which are not too hard. The operation takes very little longer than grinding, and may be quicker in many cases. The surface can be maintained plane, and the progress of the removal of material followed more easily. For the roughing stage, a 12-inch single-cut flat file should be used; an 8- or 10-inch single-cut file is useful for finishing this stage of surfacing.

The file is placed on the table, and the specimen is held flat upon it and drawn toward the tang, then lifted off. The sample must be gripped very tightly with the fingers, though it is not pressed very hard against the surface of the file. The file acts as a plane, to remove the metal with a minimum of deformation. The filings must be removed at almost every stroke, particularly if very plastic metals are being prepared, or they will clog the teeth of the file and score the sample deeply; striking the file edgewise against the work table is usually sufficient, though it is well to have a file cleaner (" file card ") at hand, and to oil or chalk the file if necessary. The filing operation should be continued several strokes after the last trace of the saw cuts has disappeared, in order to remove the underlying distorted metal; all file marks should run parallel on the surface.

If a faceted or rounded surface is formed from either the grinding wheels or the filing, it is usually rather difficult to render it plane. Turning the specimen so that the abrasion is parallel to the ridge between the facets aids in their removal, but it is better to guard against their development by grasping the sample very firmly to prevent its rocking on the wheel or the file, and by examining it frequently to see that planeness is being maintained. In general, the front edge of the specimen will be cut away faster, and a somewhat greater pressure may well be applied to the back edge to compensate for this.

The next stage of surfacing is carried out on emery papers. To avoid rounded or faceted surfaces, it is best not to mount the paper on a lap, but to hold it flat on a plane surface, and rub the specimen back and forth upon it. A piece of plate glass is a convenient base for the paper. The arm may rest on the table, and the sample should be held firmly and pressed lightly against the abrasive surface, while being moved to and fro in a straight line for about 6 inches. The scratches are kept parallel, and in a direction crosswise of those from the grinding wheel or file. At each stage, the surfacing is continued somewhat longer than is necessary to remove the previous scratches; the sample is turned 90° in passing from one paper to a finer one.[43] Care should be taken to keep the finer papers free from particles of grit from the coarser grades.

From the finest emery paper the specimen is transferred to a horizontal metal lap covered with wool broadcloth, which is well charged with No. 600 "Carborundum" flour and kept moistened with water. The lap should not be so wet that the abrasive is washed out, and for the same reason the speed should not be more than about 400 r.p.m. The specimen is moved in and out radially; the scratches from the emery paper should be crosswise of the direction of the abrasive grains on the wheel. After most of the residual scratches have been removed, the sample may be slowly carried around the wheel, so as to be abraded from all directions. This prevents one-sided action at the edges of cavities or spots of hard or soft material. The final scratches should be very fine and parallel.

This stage may give sufficiently good surfacing for low power examinations, but further treatment is usually necessary. For ordinary work, up to 1000×, the authors prefer floated emery to rouge or other soft abrasives. This material is prepared from fine emery flour,[44] which has been levigated in a Le Chatelier apparatus or otherwise graded to such fineness that it will not settle more than 6 inches through water in three hours. The emery flour is applied, in the form of an aqueous suspension, to a lap covered with wool broadcloth; it may be shaken on from a dropping bottle. In order to avoid contamination by grit from the preceding lap the sample and the fingers should be thoroughly washed before the final stage is begun. The specimen is moved on the lap as described above. It should be examined frequently to be sure that it is really wetted by the suspension of abrasive grains, and not merely burnished by dry wool fibers.[45]

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[43] The authors have found the emery papers manufactured by the Manning Abrasive Co., Troy, N. Y., much superior in uniformity and evenness of coating to the imported papers formerly so widely recommended. As an initial stage 1 G paper is suitable; it is followed by 0, 2/0, 3/0, and 4/0 if desired, though this last stage is hardly necessary.

[44] Such as the grade SF 14× of the Washington Mills Emery Co., North Grafton, Mass., which is fine enough for use directly on many materials.

[45] The surfacing operations described above are substantially equivalent to those recommended in the most recent books on metallography; a full discussion of methods is given Appendices I and III of Sauveur's *Metallography and Heat Treatment of Iron and Steel* (McGraw-Hill Book Co., New York, 1926).

See also Campbell: *Chem. Met. Eng.* **26**, 1163 (1922).

A. S. T. M: *Metallographic Testing of Iron and Steel.* **E3–24**; *Metallographic Testing of Non-Ferrous Metals and Alloys.* **E5–27.**

Bur. Standards. Circ. **113**, p. 23.

Greaves and Wrighton: *Practical Microscopical Metallography* (Chapman and Hall, London, 1924), Chap. II.

Williams and Homerberg: *Principles of Metallography* (McGraw-Hill Book Co., New York, 1928) p. 30.

Pirk: *Gen. Elec. Rev.* **28**, 573, 800 (1925); **30**, 160, 264 (1927).

When the last trace of scratches from the No. 600 " Carborundum " lap has been removed, the sample is lifted from the lap rinsed in water and dried carefully with clean lens paper. The slightest grease or finger mark will interfere with the etching operations which usually follow examination of the polished surface.

The various operations which have been described require only a very few minutes each, so that most metals can be carried through the entire surfacing procedure in about 15 minutes. Pulsifer shortens this time somewhat by rubbing the sample directly on a heap of moist abrasive;[46] he depends almost as much on deep etching as on abrasion, for the final surface.

For examinations at very high magnifications, the last stage of surfacing may be carried out by means of magnesium oxide instead of emery, according to the method of Lucas.[47] Silk broadcloth is used as a cover for the lap, which is heavily charged with abrasive and run at a rather low speed. The cloth should be kept moist when not in use, and washed out if left over night, to prevent the formation of gritty particles of magnesium carbonate. Guthrie[48] recommends a lap coated with solid paraffin and charged with a suspension of the abrasive in a glycerine-soap solution.

Very plastic metals, such as lead, tin, aluminum, and their alloys, are very subject to surface flow at all stages in their preparation, and tend to glaze the emery papers with a film of metal, even when very light pressure is applied. Grains of abrasive may also be picked up and embedded in the specimen. Lubrication of the paper with paraffin in kerosene will eliminate this. Vilella and Beregekoff[49] describe the procedure in detail; they recommend a soapy suspension of alumina as the final stage. If any oily material is used as a lubricant, it should be completely removed if the sample is to be etched, or the results may be anomalous and misleading.

Any surfacing operation which is carried out on a sample containing constituents of dissimilar hardness will tend to abrade the softer material more rapidly, and to leave the harder material standing out in low relief. If the abrasive is borne on a soft, springy surface this effect may be accentuated as " relief polishing," for the purpose of revealing different degrees of hardness in the specimen. Cloth laps have a distinct tendency to give a surface of this type, and, indeed, may be wholly unsatisfactory for work on specimens where no relief is desired. If the effect of non-uniform hardness in the specimen is to be minimized, as much of the surfacing as possible should be done on the emery papers, including the 4/0, on an oil or water stone, or on plate glass or flat metal charged with the abrasive powder. If the cloth laps are used at all, it must be for a minimum period. Special precautions are necessary in preparing specimens for the study of non-metallic inclusions in metals, to avoid the loss of the included particles, or the production of false appearances from improper surfacing.[50]

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[46] *Chem. Met. Eng.* **29**, 838 (1923).
Amer. Inst. Min. Met. Eng. Techn. Pub. **137–E.**
Mining and Metallurgy, Oct. 1928.

[47] Sauveur: *op. cit.* Appendix III, pp. 525–8.

[48] *Trans. Amer. Soc. Steel Treat.* **7**, 337–62 (1925).

[49] *Ind. Eng. Chem.* **19**, 1049 (1927).

If the nature of the *surface* of a specimen is to be studied, by means of a section normal to this surface, care must be taken not to bevel the edges or to round them off in polishing. Platings, surface carburization or corrosion, and other superficial alterations require such examinations. It is best to deposit a layer of nickel or copper on the sample, before cutting through it;[51] this coating will protect the edges from being abraded too rapidly. If electrodeposition of a protecting layer is undesirable, a plastic metal may be pressed in contact with the surface, as is done in the study of zinc coated steel,[52] or a low melting alloy may be cast around the sample, and sectioned and surfaced with it.[53] For careful examinations of the extreme outer portion of the surface the protecting material should have as nearly as possible the same hardness, and particular care should be taken to prevent any relief polishing, and to avoid scratches parallel to the surface at any stage.

Very small samples which are inconvenient to hold or to surface may be embedded in low melting alloys such as Wood's or Rose's metals, in sealing wax, in "Bakelite"[54] or other cement, or may be held in metal clamps if they are in sheet or wire form.[55]

The methods of surfacing which have been described in the preceding pages have been developed primarily for metals. They are applicable, with possible modifications, to other reasonably tough and coherent materials, but if very brittle or friable substances are to be surfaced somewhat different procedures are necessary. The cloth-covered surface of the laps is too springy for work with vitreous or easily cleavable specimens, so the abrasive is usually carried on a hard surface. Plate glass is satisfactory for the preliminary stages; cast iron, bronze, or block tin laps may be used. Various grades of emery, " Carborundum " or "Alundum" flour may be used, lubricated with water, soap solution, paraffin oil, or turpentine. The lap should be thoroughly true, to avoid pounding, and should run at a relatively slow speed, 100–400 r.p.m. Fine jeweler's rouge, levigated alumina, or putty powder (tin oxide) may be employed for the final stage.

For rough examinations of the structure of relatively transparent material, either by inclined or transmitted illumination, carefully prepared surfaces may not be necessary. By the use of a mounting medium of the proper refractive index, surface imperfections may be rendered invisible, and the interior features more clearly revealed. Merely covering the surface with a film of liquid is frequently almost as satisfactory as fine polishing, and is particularly useful for preliminary studies of the progress of surfacing operations on transparent or translucent specimens.

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[50] Comstock: *Iron Age* **115**, 1185 (1925).

Wohrman: *Trans. Amer. Soc. Steel Treat.* **14**, 81, 255, 385, 539 (1928).

[51] Rawdon: *Chem. Met. Eng.* **24**, 475 (1921).

U. S. Bur. Standards Circ. **113** (1922), p. 24.

[52] Finkeldey: Microstructure of Zinc Coatings. *N. J. Zinc Co. Research Bulletin.*

Proc. A. S. T. M. **26**, Part II (1926).

[53] Campion and Ferguson: *Jour. Iron and Steel Inst.* **88**, 385 (1913).

[54] Head: *Eng. Min. Jour. Press.* **119**, 889 (1925).

Short: *Econ. Geol.* **21**, 648 (1926).

[55] Several such methods are illustrated in the publications by Finkeldey and by Lucas, just cited.

The principal difficulty in dealing with brittle specimens appears to lie in the early stages of surfacing. Violent treatment in cutting or grinding the sample may cause incipient cracks which spread and weaken it as the operation proceeds. If a saw is used for cutting off the piece it should have fine teeth and should be used with very little force. Jeweler's hacksaws (Fig. 77) are very useful, especially in sectioning weak and crumbly material such as small arms primers,[56] match heads, enamel coatings on metals, etc. The teeth should be fine, 20–30 per inch, and the blade tough and flexible. Materials too hard to be cut by a hacksaw may be sectioned by a diamond or " Carborundum " saw, or a fractured surface may be used. In any case, it may be desirable to carry out the first grinding operation by hand on a rather fine " Carborundum " stone, lubricated with oil or water. After the surface has been rendered substantially plane without chipping or cracking, the later stages on the laps are much less likely to cause trouble. If the specimen is very prone to chip or crumble, grinding in a single direction will sometimes help to prevent fragments from chipping out. Filling up cavities with "Bakelite," Canada balsam, or other cement will help to support their edges and to prevent large fragments from coming off to score the rest of the specimen.

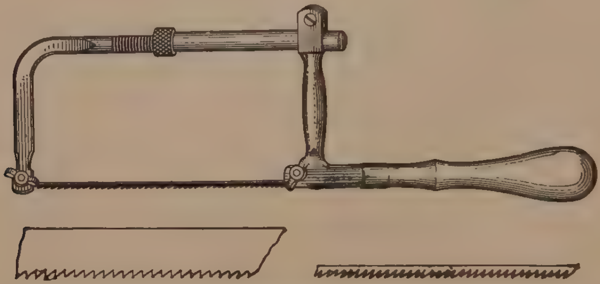

FIG. 77. Jeweler's Hack Saw. Sections of blades are shown about twice actual size.

Occasionally it is necessary to surface materials which are affected by even a brief contact with water. The procedures outlined above may be employed, if alcohol, kerosene, or a petroleum oil such as " Nujol " is used to suspend the abrasive and to lubricate the specimen. If the sample is readily attacked by the atmosphere, it may be well to keep it covered with the oil until it is examined.[57]

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[56] Chamot: *Microscopy of Small Arms Primers* (Ithaca, N. Y. 1922), p. 35.

[57] Hume-Rothery: *Jour. Inst. Metals* **40**, 65–83 (1928).

After the specimen is surfaced by one of the procedures which have been described, it is ready for microscopic examination by vertical or inclined illumination. Since in many cases materials which have simply been polished present no great differences in color or reflecting power, some sort of etching procedure is frequently necessary. Etching should not be employed until after the sample has been thoroughly studied in the unetched condition, for otherwise inclusions and other structural features may be masked.

Etching of surfaced specimens which are to be studied by reflected light is commonly employed to differentiate their various ingredients. The choice of etching reagent depends upon the chemical nature of the materials; it may act simply as a selective solvent, it may oxidize certain constituents more rapidly than others, it may give a deposit of colored material, it may attack the boundaries of crystal grains, or it may develop on them microscopic pits which cause oriented luster.

In the case of metals and alloys the etching procedures are more or less standardized, and practically all of the works which have been referred to include formulas for etchants for a number of different alloys. For ordinary general work, the following are satisfactory:

Ferrous alloys: 2 per cent HNO_3 in alcohol.
4 per cent picric acid in alcohol.
2 g. picric acid, 98 cc. of 25 per cent NaOH in water.
Copper alloys: 25–50 per cent HNO_3.
25 g. CrO_3, 15 g. Na_2SO_4, 100 cc. H_2O.
10 cc. NH_4OH, 1 cc. H_2O_2.
Aluminum alloys: 10 per cent NaOH in water.

Tin and lead alloys: 5 per cent $AgNO_3$ in water. Wipe off deposit of silver with the finger, under running water.

Before being etched, the polished surface of the specimen should be studied carefully for any indications of inclusions, relief polishing, or localized differences of color. The surface should be thoroughly clean and free from grease. It may then be etched by being dipped in the proper reagent for a few seconds, being moved about or swabbed meanwhile to dislodge any bubbles which might form. The sample is then washed, dried, and examined. In general, it is better to etch very cautiously at first, repeating if necessary until the desired structure is revealed. If the specimen is over-etched, it must be repolished, at least through the last two stages. When the etching is complete, the piece should be very thoroughly washed in running water (preferably warm) and dried with lens paper. Some workers prefer to rinse off the water with alcohol and to dry in an air blast.

If surface flow has occurred in the preparation of the metal, a thin film may be formed which does not etch normally and which possesses no significant structure. This may be etched away, and the specimen repolished lightly and re-etched lightly. Etching is the only sure way of completely removing surface films, but it should not be necessary to depend on it for this purpose if the specimen is properly polished, unless very soft metals such as lead and tin are dealt with.

It is often desirable to etch a sample by more than one reagent, repolishing each time, in order to reveal different constituents and to check the interpretation of the structure. Considerable practice is necessary for proper application of the various reagents to give best results in all cases.

A great variety of materials are capable of study by surfacing according to methods more or less similar to those which have been outlined. Some typical and suggestive examples are as follows:[58] carbon and graphite products,[59] gums and resins,[60] cement clinker,[61] concrete,[62] vitreous enamels on metals,[63] and refractories.[64] Perhaps the greatest field outside of metallography proper is in the study of ores and opaque minerals in general.[65]

Preparing thin sections of hard material, for study by transmitted light, is carried out by grinding operations which are closely analogous to those already described. Occasionally a fractured fragment may be obtained which, at least in places, is sufficiently thin and transparent for study, but generally the specimen has to be ground smooth on one face, and then another surface has to be created parallel and very close to the first one.

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[58] White and Thiessen: *Bur. Mines. Bull.* **38**, pp. 187–378.
Turner: *Trans. Amer. Inst. Min. Met. Eng.* **71**, 127 (1925).
Seyler: *idem,* p. 117.
Thiessen: *idem,* p. 35.
Wahl and Bayard: *Comptes rendus* **157**, 380 (1913).

[59] Rousch: *Ind. Eng. Chem.* **3**, 368 (1911), Ramdohr: *Arch. Eisenhüttenw.* **1**, 669 (1928).

[60] Nicolardot and Coffignier: Chim. et. Ind. **11**, 456 (1924). Stock: *Farben Ztg.* **30**, 2340, 2407, 2475, 2542, 2604, 2668 (1925).

[61] Desch: *Concrete Cement Age* (3), **3**, 27.
Hattori: *Rept. Ind. Dept. Cent. Res. Lab.* (Formosa) **1** (1922).

[62] Johnson: *Eng. Rec.* **71**, 98, 160, 263, 301, 320 (1914).

[63] Comstock: *Jour. Amer. Ceram. Soc.* **6**, 873 (1923).

[64] Desch: *Trans. Faraday Soc.* **12**, 1511 (1917).

[65] In addition to the references given on page 157, the following may be mentioned, as dealing primarily with preparation methods as applied to opaque minerals:

Head: *Eng. Min. Jour. Press.* **119**, 889 (1925).
Thompson: *Can. Min. J.* **43**, 78 (1922); *Trans. Can. Inst. Min. Met.* **29**, (1926).
Short: *Econ. Geol.* **21**, 648–64 (1926).

The technique of this procedure has been developed chiefly by petrographers, for the preparation of thin sections of rocks, and most methods are adaptations of those described in the standard works on this subject.[66] A very complete discussion of the entire operation is given by Keyes;[67] it should be studied by every worker who attempts to acquire the difficult technique that is necessary.[68]

A slice of the material a few millimeters thick is cut by a diamond saw, or a fragment of convenient size is chipped off by a hammer. On account of the relative brittleness of most non-metallic hard substances it is usually best to surface them according to the methods given on page 159 for brittle materials. One face of the sample is ground plane on a stone, or on a metal lap charged with fairly fine "Carborundum" flour (Grade F) and kept wet with water. It is washed free from grit, and surfaced further on a second lap carrying finer "Carborundum" (No. 400 or 600). For most work this constitutes the last stage of grinding, but a final polishing with very fine levigated emery or "Carborundum" on a glass plate may be desirable in the case of material possessing fine detail.

After washing and drying thoroughly, the sample is placed, smooth surface down, on a microscope slide on which some "cooked" Canada balsam has been melted by means of a hot plate. The balsam is pressed out to a thin film between the slide and the specimen; no bubbles must be present. The preparation is then allowed to cool, and the rough grinding operation is repeated on the other face of the sample. This must be thinned down until the edge of the layer of balsam is reached, when surfacing by the finer grade of "Carborundum" is begun. The last reduction in thickness and final surfacing may be carried out by hand, on a glass plate with levigated emery. Care must be taken to keep the section uniform in thickness and not wedge-shaped, and to avoid tearing out large fragments which may score the surface deeply. The ultimate thickness will depend upon the nature of the material and the skill of the operator; most rock sections are 0.03 mm. thick.

The progress of the grinding of the section should be followed by means of microscopic examinations, in order that any tendency of the material to crumble or loosen from the slide may be detected, and so that the necessary thinness may be recognized. Covering the surface with liquid of approximately the same refractive index as the specimen will render it transparent even though incompletely polished; the liquid should be chosen to have no effect upon the material or upon the cement used.

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[66] Johannsen: *Manual of Petrographic Methods* (McGraw-Hill Book Co., New York, 1918), Chap. 41.

Holmes: *Petrographic Methods and Calculations* (Murby, London, 1921), pp. 231–49.

Winchell: *Elements of Optical Mineralogy, Part I* (John Wiley & Sons, New York, 1922), Chap. VIII.

Lee: *Microtomist's Vade Mecum* (P. Blakiston's Son, Philadelphia, 1928), pp. 110, 473, discusses methods for preparing thin sections of bones, teeth, and similar material, by grinding.

[67] *Amer. Jour. Sci.* **10**, 538–50 (1925); *Geophysical Laboratory*, Reprint **577**.

[68] Thin sections of hard materials of all sorts are prepared to order by W. Harold Tomlinson, 114 Yale Ave., Swarthmore, Pa.

When a sufficiently thin section has been prepared, the slide is heated on a hot plate, a small amount of balsam is melted on the upper surface of the specimen, and a cover-glass is applied and pressed down. The section may be transferred and mounted on another slide if desired, but this increases the risk of breakage. If chemical tests are to be applied to the specimen, the surface is left uncovered.

Very porous or friable materials will probably disintegrate under the above treatment, and must be supported in some manner during the surfacing operations. The usual procedure is to heat in molten balsam until all interstices are filled; a solution of balsam in ether may be used and allowed to harden by evaporation at room temperature. The reinforced sample is then ground down and surfaced by the methods given. "Bakelite" has been employed with success by Ross[69] and others;[70] the varnish (diluted with ether, acetone or methyl alcohol if necessary) is allowed to soak into the sample for 1 to 4 days. The solvent is evaporated, and the "Bakelite" hardened by heating for one or two days at 70–90° C. Higher temperatures shorten the time required for curing the "Bakelite," but may alter the specimen; lower temperatures (50° C) will harden the "Bakelite" in the course of a week or more. Balsam or "Bakelite" may be used for cementing to the slide for grinding.

If the substance to be sectioned is affected by water, the grinding operations must be modified by the use of oil as a suspending medium for the abrasive. Thin sections of soluble salts may be prepared in this way.[71]

The various procedures outlined above, with appropriate modifications, may be applied to the preparation of thin sections of a great variety of hard and brittle materials; a few typical examples are as follows: abrasive wheels,[72] coal,[73] refractories,[74] porcelain[75] and earthenware,[76] cement clinker,[77] setting of cement,[78] glass and ceramics,[79] and charcoal.[80]

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[69] *Amer. Jour. Sci.* **7**, 483 (1924); *Econ. Geol.* **21**, 454 (1926).

[70] Report of the Stone Preservation Committee, *Dept. Sci. Ind. Res.* (London, **1927**).

Legette: *Jour. Geol.* **36**, 549 (1928).

[71] Korreng: *Centr. Min. Geol.* **1913**, 408.

[72] Milligan: *Ind. Eng. Chem.* **19**, 1127 (1927).

[73] White and Thiessen: *Bur. Mines. Bull.* **38**.

Lomax: *Jour. Roy. Micros. Soc.* **280**, 239 (1927).

[74] Fearnsides: *Trans. Faraday Soc.* **12**, 148 (1917).

Insley and Klein: *Bur. Stds. Tech. Paper*, **124**.

Rigg: *Ind. Eng. Chem.* **5**, 549 (1913).

Boericke: *Min. and Met.* **10**, 16 (1929).

[75] Klein: *Bur. Stds. Tech. Paper*, **80**.

[76] Insley: *Jour. Amer. Ceram. Soc.* **10**, 317 (1927).

[77] Glasenapp: *Chem. Ztg.* **37**, 522 (1913).

Apparatus for grinding surfaces and sections is described in many of the larger works which have been cited.[81] The chief requirements are: adequate range and variation of speed; absence of play or vibration of laps; separate laps for each grade of abrasive; drains for liquid thrown off from laps; covers to protect laps from grit; easy removal of laps for cleaning or recovering. The Warner and Swasey apparatus[82] and the Guthrie-Leitz apparatus[83] are the most satisfactory of the larger outfits. Smaller grinding apparatus with one or more laps or abrasive wheels is obtainable from a number of dealers.

MOUNTING SPECIMENS FOR MICROSCOPICAL STUDY

Some objects are of such size and shape that they may be placed directly upon the stage of the microscope and examined forthwith. No mounting procedure is necessary for polished metal specimens which are to be examined with an inverted-type microscope. The Greenough binocular microscope and similar low power instruments do not necessitate any special means of holding or supporting the sample in many cases. In general, however, somewhat less simple treatment is required.

The mounting of microscopical specimens serves a number of purposes: The sample is disposed in a plane perpendicular to the axis of the microscope; its arrangement is made uniform; its visibility is improved; it is protected from dust and mechanical or chemical disturbance; the proper functioning of high aperture objectives and condensers is made possible.

Opaque specimens which have been surfaced require simply to be arranged normal to the axis of the microscope, so that all parts can be brought in focus simultaneously. This is easily accomplished by embedding the piece in a lump of "Plasticene" or modeling wax, on a microscope slide or a flat plate of metal. The upper surface of the sample can be placed practically per-

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[78] Shepherd, Rankin, and Wright: *Ind. Eng. Chem.* **3**, 211 (1911); *Amer. Jour. Sci.* **39**, 1 (1915).

Shepherd, Day, and Wright: *Amer. Jour. Sci.* **28**, 293 (1909).

[79] Peck: *Jour. Amer. Ceram. Soc.* **2**, 695 (1919). Schulz: *Mikrokosmos* **19**, 217 (1925–6).

[80] Clerc: *Zeits. wiss. Mikros.* **44**, 417 (1927).

[81] Sauveur: *op. cit.* Appendix I.

Johannsen: *loc. cit.*

[82] Obtainable from the Chemical Rubber Co., Cleveland, Ohio.

[83] Obtainable from E. Leitz, Inc., New York.

pendicular to the axis of the microscope by trial, but the leveling cup shown in Fig. 78 is more convenient.[84]

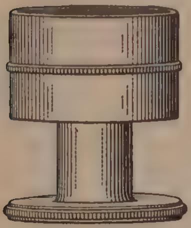

FIG. 78. Leveling Cup for mounting Opaque Specimens.

Transparent materials are handled differently, since their microscopic image is largely due to refraction rather than to reflection. It may be sufficient to lay a sample on a glass slide, and to examine it by transmitted light; little more is necessary in the study of sheet celluloid or viscose, ground or frosted glass, and similar flat objects. If the specimen presents any marked irregularities of surface, or is fragmental or fibrous, a mounting medium is usually required.[85]

The function of the mounting medium in controlling the refraction image of transparent materials has already been emphasized (page 76). It is only by reducing the opacity of the shading of the object, which is caused by reflection and refraction at its surfaces, that sufficient transparency may be imparted to the preparation to permit study of its interior features. Maximum transparency is produced if the specimen and the mountant have the same index of refraction, and under these conditions surface characteristics are practically invisible, and inner structures are clearly revealed. This procedure is valuable in the microscopic or ultramicroscopic examination of fragments of solids containing inclusions, the interior details of textile and paper fibers or starch grains, roughly surfaced thin sections, and any other substances where emphasis is not to be placed upon surface character. Colored materials may well be examined in a medium of refractive index near their own in order to produce a true color image instead of a mixed color and refraction image.

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[84] This simple apparatus can be made in any machine shop, or a device serving the same purpose can be obtained from Leitz. It consists of a flat-topped plunger which moves up or down in the outer cylinder by means of a screw. The top of the plunger is covered by several thicknesses of lens paper, on which the polished face of the specimen is laid. The microscope slide bearing the "Plasticene" is placed across the rim of the cylinder, which is adjusted so that the specimen is pressed firmly into the plastic mountant. Since the rim of the cylinder and the top of the plunger are parallel, the surface of the sample and the slide on which it rests will be parallel also, and when placed upon the stage of the microscope will be normal to its axis.

[85] The methods of preparing and mounting crystalline material by recrystallization operations are discussed in Chapter X.

If the exterior of the object is the subject of investigation, a mounting medium of distinctly different refractive index should be selected; it may be either higher or lower than that of the object.[86] By this means fine details on the surface are made more prominent, and the whole image presents greater contrast. Mounting in media of considerably different refractive index is important in the study of the surface of glass, plastics, waxes, fibers, crystals, and in the examination of pigments consisting of very fine particles.

The **properties of an ideal mounting medium** for transparent materials are:

1–Transparency and absence of color, at least in thin layers.

2–Refractive index different from that of the object, but not enough to cause too heavy shading in the image.

3–Sufficient fluidity to permeate the specimen thoroughly and to permit the escape of entrapped air.

4–Sufficient viscosity to hold the cover-glass and the fine structures of the object in place.

5–Chemical inertness and lack of solvent action on the object, the glass or the sealing material.

6–Permanence; non-volatile, and non-hygroscopic.

7–Ease of application, at moderate temperature.

8–Miscibility with water, for mounting imperfectly dried material.

9–Refractive index high enough to utilize full aperture of condenser and objective.

It is obvious that no one medium can possess all of these properties, but a number of substances are reasonably satisfactory in most respects.[87] Water ($n = 1.33$) is very commonly employed, but is suitable only for brief examinations. Textile fibers are swelled considerably by it, and soluble materials are extracted from mixtures. Alcohol ($n = 1.37$), xylene ($n = 1.49$), benzene ($n = 1.49$), or bromoform ($n = 1.57$) are useful for temporary mounts, since they cover a wide range of refractive indices, and are quickly and completely removable by evaporation. Glycerine ($n = 1.46 +$) is too hygroscopic for permanency, but is useful for temporary mounts which must not dry out during the examination.

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[86] The difference between the refractive indices of the object and the surrounding medium has been called the "index of visibility." See Spitta: *Microscopy* (1920), p. 497.

[87] Detailed discussions of various mounting media are given in connection with sectioning methods already cited. See also Lee: *op. cit.* Chap. XIX; Jones: *Photog. Jour.* **54**, 14 (1914); Denham: *Jour. Roy. Micros. Soc.* **263**, **190** (1923); Kolbe: *Zeits. wiss. Mikros.* **44**, 196 (1927).

Glycerine jelly (n = ca. 1.47) is very useful for mounting imperfectly dry material, such as textile or paper fibers. It is melted in a water bath, and hardens on cooling. The edges of the preparation should be sealed. Glucose syrup (" Karo ") (n = ca. 1.47) is similarly useful, but does not harden much from evaporation and does not hold the cover-glass firmly in place; it is useful for semi-permanent mounts.

" Euparal "[88] (n = 1.48+) is a preparation of resins which is very useful for mounting textile fibers on account of its refractive index and the fact that it does not require perfect dehydration of the sample. It hardens by evaporation, but never becomes as brittle as Canada balsam.

Dammar (n = 1.52) may be employed as a syrupy solution in xylene, or applied in the melted condition. It is preferably purified by dissolving in chloroform, filtering, precipitating the waxy constituents by dilution with alcohol, and evaporating the solution at low temperature.

Canada balsam (n = 1.53) in xylene solution is widely useful for mounting dry materials. No moisture should be present in the sample. If the balsam is too dilute, evaporation of the solvent will cause marked shrinkage, and air bubbles will be drawn into the preparation at the edges of the cover-glass.

Styrax (n = 1.57–1.6) varies greatly in its index of refraction but in general is useful where a highly refractive mountant is required. It is handled like Canada balsam. Balsam of Peru is similar but more highly colored.

"Bakelite" (n = 1.58–1.63, depending on composition and curing) is used in the form of varnish as a mountant for friable specimens. It must be cured by heating at 70–100° C. for several hours. The color is objectionable except in very thin layers.

Piperine (n = 1.64+) serves as a highly refractive mountant. When fused and allowed to cool it crystallizes very slowly, particularly if it has previously been kept molten at a temperature of 180° C. for an hour or more.[89] The material thus prepared is liquefied by heat when used. Its pale brownish color is somewhat objectionable.

" Hyrax "[90] (n = 1.63 – 1.75+) is a synthetic mounting medium which has recently been developed. It is pale in color, and hardens by slow evaporation without becoming brittle; its refractive index meanwhile increases to the maximum value.

The various liquids given in the tables on pages 385, 386 are useful for temporary mounts, and from them material of any desired refractive index may be selected.

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[88] Obtainable from Flatters & Garnett, Manchester, England; an equivalent material is sold in America under the name of " Diaphane." Neither retains its low refractive index, that of the hardened material being about 1.53.

[89] Jones: *Watson's Microscope Record*, No. 5, p. 11 (May, 1925) states that piperine thus treated shows little or no crystallization in preparations over ten years old.

[90] *Science*, **70**, 16 (1929).

Obtainable from Penn & Ruedrich, Box 26, Associated, Calif.

The color of the mounting medium is sometimes of considerable importance. In photomicrography, especially with blue or ultraviolet light, even a moderately yellow mountant may appear relatively opaque. Colored mounting media are occasionally employed to increase contrast, especially when the object itself is colorless, difficult to stain, or has a refractive index near that of the mounting material. Under such conditions an absorption image is obtained, in which the object appears light against a colored or shaded background.

Mounting media are usually applied by placing a drop of the liquid medium in the center of the slide, and introducing the specimen into it. All parts should be immersed, and distributed in the position desired; any bubbles which escape may be punctured before the cover-glass is put on. The cover-glass should be placed carefully upon the surface of the drop, and allowed to settle down without including any air. It may then be pressed cautiously, to expel the excess mounting medium, and to render the preparation thin and as nearly in one plane as is possible without distortion. Spring forceps may be used to hold the cover-glass in place until the mountant hardens. The quantity of medium should be barely enough to fill the space under the cover-glass, and an excess should be avoided, if possible. If some has been squeezed out at the edges of the cover-glass it may be carefully removed by scraping, if brittle, or by a bit of filter paper moistened with an appropriate solvent.

Temporary mounts in non-volatile media require no further preparation. If the mountant is likely to evaporate before the examination is complete, it is useful to seal the edges of the cover-glass by vaseline or paraffine. If round cover-glasses are used, they may be " ringed " with the melted sealing material, by means of a fine brush and a revolving turntable.

For permanent mounts it is advisable to seal the edges of the cover-glass with a cement such as shellac, Brunswick black, " Duco," or other varnish.[91] The surfaces should be thoroughly clean in order that good adhesion will be possible. Several thin coats are better than one thick one, and enough cement should be applied to give a substantial layer at the edge and in the angle of the cover-glass. Once sealed in this way, the specimen may be used with immersion objectives and wiped clean with no risk of displacing the cover-glass.

Powdered materials, such as pigments, fillers, and abrasives, should be mounted in a medium which is chosen to give good contrast. The finer the pigment and the lower its refractive index, the more important this is; water mounts may be necessary, and in some cases sufficient visibility is obtained only with dry preparations. It is also essential that the particles should be evenly distributed, and not heaped several deep or flocculated into clumps. Viscous mounting media, or deflocculating agents if water is used, may be necessary to permit a uniform suspension of the material before it is spread out on the slide. After the particles are thoroughly dispersed by careful stirring in a drop of the mountant, the cover-glass may be applied. The layer of material should be pressed as thin as possible, to render the particles as nearly in the same plane as their varying diameters permit.[92] Since fine powders cannot be evenly distributed when dry, it is sometimes desirable to disperse the particles in water or some other volatile liquid,[93] which may be evaporated to give a dry mount, or to permit the use of a medium which has poor dispersing power. Examination of the particles in the vehicle in which they are ultimately to be used (linseed oil, lacquer, rubber, etc.) is also to be recommended, since their tendency to flocculate or be dispersed may be different in different media.

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[91] See also Lee: *op. cit.* Chap. XX.

In the case of materials which flocculate badly, or which require dry mounting for maximum visibility, it is advisble to follow Green's method of distributing the particles evenly.[94] A drop or two of a suspension of the powder, in a volatile liquid which disperses it well, is spread in a film on a smooth slide, by stroking with a glass rod held flatwise. The "rubbing out" is continued until practically all of the liquid has evaporated, in order to prevent flocculation. This method is suitable for particles which are very fine and uniform in size, but is likely to cause segregation if particles of various sizes are present. When the grains have been spread out with as little clumping as possible and the distributing liquid has been evaporated, any mounting medium may be employed, provided care is taken not to dislodge the particles from the slide to which they tend to cling.

Powdered materials having wide particle-size range may be mounted in liquid, by preparing a uniform suspension and pressing it into a film beneath a cover-glass. Settling of the coarse particles is likely to prevent even distribution, and Brownian movement of the fine particles is usually troublesome. The use of a sufficiently viscous mounting medium obviates these difficulties, and prevents the flocculation of the dispersed particles. Since the layer of suspension must necessarily be as thick as the coarsest particles, the finer ones will be distributed at different levels; the use of objectives of high resolving power and little depth of focus is thereby hindered.

Spreading out a suspension of non-uniform particles in a mobile and volatile liquid, preparatory to dry mounting, is unsatisfactory on account of the risk of segregation or crushing the coarse particles and flocculation of the fine ones before evaporation of the dispersing medium is complete. By the use of a collodion solution, which is of suitable viscosity when wet and which evaporates rapidly to leave a minimum of solid residue, these sources of error are eliminated. Most powdered materials are readily dispersed in a collodion solution having the consistency of thin syrup. A drop of the suspension is flowed or spread out in a thin layer on a slide, where it almost immediately sets to a gel by evaporation. The particles are thus fixed in place, and on further evaporation the layer of collodion gel decreases in thickness, so that the particles are all brought substantially into the plane of the surface of the slide. The residual film of dry collodion, not more than a few microns thick, serves to anchor them so that a liquid of appropriate refractive index may be used as a final mounting medium under a cover-glass, to give a clearer outline to the particles and to permit the use of immersion objectives. If the particles are of low visibility they may be examined dry, without risk of displacement on the slide.

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[92] Perrott and Kinney: *Jour. Amer. Ceram. Soc.* **6**, 429, 435 (1923).

[93] Weigel: Size and Character of Grains of Non-metallic Mineral Fillers. *Bur. Mines. Tech. Paper*, **296**, p. 7.

[94] *Jour. Franklin Inst.* **192**, 637 (1921).
Ind. Eng. Chem. **16**, 677 (1924).

A mounting procedure[95] which is particularly valuable in the preparation of fine pigments for photomicrography employs a melted gum such as dammar or balsam. The medium is kept fluid on a hot plate while the particles are dispersed in it as uniformly as possible by stirring. The ultimate dispersing action is accomplished by the shearing forces in the liquid, when the suspension is pressed to a very thin film beneath a cover-glass. The mountant is hardened by cooling, so that the particles are permanently prevented from flocculation or Brownian movement and lie substantially in one plane.

Fibrous materials should be spread out as uniformly as possible; most raw textile fibers must be "teased apart" with dissecting needles after softening in water, or in hot 1 per cent NaOH solution. Papers or felts should be thoroughly pulped by similar means,[96] and spread out on the slide, avoiding clumping as much as possible. Water mounts are adequate for most work. Glycerine jelly or "Euparal" are suitable for permanent preparations.[97]

Food stuffs, drugs, and other animal and vegetable materials may require treatment with clearing or macerating agents which increase the transparency or visibility of their structures, and to render them more separate and distinct.[98]

Staining of microscopical preparations is often used to give information that is not obtainable from a refraction image of a colorless specimen. Careful preliminary study of the unstained object, in various mounting media, and under different conditions of illumination, should always be carried out, and the microscopist should not form the habit of depending upon stained specimens for all his observations. Color images are frequently far inferior to refraction images, as regards ease of interpretation of appearances, and the application and behavior of stains may not be consistent in the case of novel materials. Staining procedure is chiefly useful for the following purposes:

1–Increasing the visibility of specimens, as a substitute for a mounting medium of the proper refractive index.

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[95] Originated in the Research Laboratories of the New Jersey Zinc Co.

[96] T. A. P. P. I.: *Manufacture of Pulp and Paper*, Vol. 5 (1925), p. 49. A. S. T. M.: *Method of Analysis of Roofing Felt*, **D 272–27T.**

[97] General methods, applicable to the preparation of various kinds of fibers for microscopic examination, are given by Lawrie: *Textile Microscopy* (1928), Chap. IX; and Hausman: *Amer. Jour. Anat.* **27**, 463 (1920).

[98] Lee: *op. cit.* Chaps. VI, XXIII.
Wallis: *op. cit.* Chaps. V and VI.

2–Revealing chemical differences between various portions of the specimen.

An enormous number of stains and methods of applying them are given in the literature on plant and animal histology.[99] Most of the stains used are organic dyestuffs, which are adsorbed more or less selectively on the different kinds of material which constitute the object. Certain stains act more like chemical reagents and may be used as more or less specific tests *in situ*.[100] Perhaps the most generally useful of these are the stains employed for paper and other cellulose materials.[101]

> Of the various stains in use, that of Herzberg is most widely applicable, and, on account of its selective coloring, enables fibers having different origins or treatments to be recognized in mixtures at a glance. This is particularly valuable in estimating quantitatively the composition of the pulp.
>
> The formula for making Herzberg's stain is given on page 458.

Slides and cover-glasses for mounting microscopical objects should be chosen primarily for their thickness and uniformity. They serve to protect the specimen, and to render it easily visible by enclosing it between plane parallel surfaces, so that it is substantially in one plane and is completely surrounded by the mounting medium.

Slides should be from 1 to 1.5 mm. thick and — for most work — of the full dimensions, 25 × 75 mm. Half slides may be purchased, or prepared by cutting these in two. The glass should be chemically resistant, especially if reagents are to be used on the surface of the slide; the slightly greenish glass from which the harder and more insoluble slides are made is not noticeably colored except when viewed edgewise, and is much more permanent than the soft colorless glass which is also on the market.

Slides with concave depressions ground and polished in the upper surface are useful for handling liquids of low surface tension, or for thick objects which are difficult to immerse completely in the mounting medium. Thicker slides, with deeper cavities, may also be obtained, or glass rings may be used to surround thick objects and to support the cover-glass to form a cell. Slides of large size, 50 × 75 mm., are useful when powdered materials are spread out for hand sorting.

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[99] Gage: *The Microscope* (Comstock Pub. Co., Ithaca, N. Y., 1925), p. 392.
Kingsbury and Johannsen: *op. cit.* pp. 37–55.
Lee: *op. cit.* Chap. XI.
Hager and Tobler: *Das Mikroskop und sein Anwendung* (J. Springer, Berlin, 1925), p. 74.
Mathews: *Textile Fibers* (John Wiley & Sons, New York, 1924), Chap. XXV.
Lawrie: *Textile Microscopy* (E. Benn, London, 1928), Chap. VIII.

[100] See A. O. A. C.: *Methods of Analysis* (1920), pp. 260–1.

[101] T. A. P. P. I.: *Manufacture of Pulp and Paper*, Vol. 5 (1925), p. 50.

Slides of transparent fused quartz may be obtained for work requiring extreme resistence to chemicals and to temperature changes; they are rather expensive if polished plane. Pyrex glass slides may, for some purposes, be used as a substitute.

For studies with hydrofluoric acid and its salts, celluloid slides will be found practicable and far more convenient than glass slides coated with varnish, Canada balsam, "Bakelite," or similar materials. Celluloid slides will stand a moderate amount of heating, but cellulose acetate is much less inflammable.

Cover-glasses may be obtained in various sizes, either rectangular or round. The latter are preferable for most work, especially if their edges are to be sealed in making permanent mounts.[102] Cover-glasses are sorted according to thickness, No. 1's being about 0.15 mm. thick, No. 2's about 0.2 mm., and No. 3's, 0.25–0.50 mm. For work with objectives of short working distance No. 1 cover-glasses should always be chosen. A small dial gage is useful for measuring the thickness of slides and cover-glasses.

Slides and cover-glass should be kept scrupulously clean; new slides should be dipped in warm chromic acid cleaning mixture, rinsed in distilled water, drained, and dried in a dust-free chamber. Cover-glasses may be washed as above and then placed in a mixture of absolute alcohol and ether, in a wide-mouth bottle, from which they are taken when needed for use. The simplest test which can be applied to a slide or cover-glass to determine its fitness for use is to note whether a drop of distilled water spreads readily upon it; if it refuses to flow, and stands up in a round drop, the surface is greasy or dirty. Slides and cover-glasses free from grease are particularly essential in mounting aqueous preparations or colloidal suspensions, carrying out crystallizations, or manipulating drops of solutions in microchemical work.

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[102] A revolving turntable is used for this purpose; a fine brush is dipped in the cement and touched to the edge of the cover-glass as it is rapidly spun around.

All cloths used in wiping slides and cover-glasses must be free from lint, sizing, and grease. Well washed muslin or lintless toweling is satisfactory, but it must be remembered that after handling it soon takes up enough greasy material from the hands to render it worse than useless. For this reason it will be found convenient to keep several small pieces at hand, and to mark them so that the hands are brought in contact with only one side and the glass with the other.

In handling slides the fingers should touch the flat surfaces as little as possible and never where manipulations are to be carried on. Cover-glasses should be handled by the edges exclusively, or by means of forceps.

Used slides may be cleaned by treatment with an appropriate solvent, followed by rubbing with a thin paste of " Bon Ami." This is allowed to dry on them as a film, and is wiped off with a clean cloth, just before use if desired. Cover-glasses are so fragile that it is hardly worth while to try to clean them if they are very badly coated with gummy or greasy material. Old slides which have become scratched or etched should be discarded.

CHAPTER VI

SPECIAL METHODS FOR INTERPRETATION OF APPEARANCES AND OBSERVATION OF PHYSICAL PROPERTIES

It is frequently the case that simple observation of an object under the microscope is insufficient for a full interpretation in terms of its actual physical properties. The experienced microscopist constantly employs variations of focus and different methods of illumination, and in most instances these suffice to yield an accurate conclusion as to the nature of the object which is represented by the image, but special technique is often required, especially in dealing with novel material, as a supplement to the routine methods of examination. It is impossible to describe all the procedures which may be applied to these problems, but a few typical and suggestive methods will be outlined in the following pages.[1]

OBSERVATIONS OF FORM

The interpretation of the two-dimensional outline of the object, as seen in plan, is not particularly liable to error. However, since an appreciable depth of focus always exists, structures above or below the plane of greatest sharpness are visible, and may be mistaken for flat portions of the object. Some very instructive experiments can be made on the character of the images of simple geometrical forms such as rectangular parallelopipeds, spheres, and cylinders. If refraction images are dealt with, the path of the light rays may be traced through the object in each case, and the origin of every shadow outline may be worked out geometrically; in color images this is not so easily possible.

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[1] The quantitative measurement of dimensions and angles is discussed in Chapter XII. The use of polarized light as a means of interpreting form and structure is given in Chapter X. The influence of various methods of illumination has already been emphasized in Chapter III. Good discussions of special methods for the interpretation of appearances are given by:

A. E. Wright: *Principles of Microscopy* (1906) Part I.
Gage: *The Microscope* (1925), Chap. IV.
Carpenter: *The Microscope and Its Revelations* (1901), pp. 427–36.
Naegeli and Schwendener: *The Microscope in Theory and Practise* (1892), pp. 189–222.
Langeron: *Précis de Microscopie* (Paris, 1925), Chap. XIV.

In general it may be said that surfaces which are approximately parallel, and not too greatly inclined to the axis of the microscope, will appear transparent, and will be invisible if smooth; their edges will appear as thin dark lines. If the surfaces are an appreciable distance apart (as in the case of the top and bottom of a tabular crystal lying on the slide) so that their bounding edges intersect vertical surfaces, the outline will still be a very thin line. This is easily observed with small crystals of sodium chloride, as they form from a drop of their mother liquor. (See Figs. 32, 131.) If, however, these edge surfaces are inclined to the axis of the microscope they will refract light to one side or the other, depending on the refractive index of the object relative to that of the surrounding medium, and will appear darker than the central portions through which light travels without such deviation. Shadows in a refraction image may therefore be interpreted as corresponding to surfaces which are strongly inclined to the axis of the microscope. The direction of this inclination is only evident from a study of the third dimension of the specimen, by focusing, sectioning, or stereoscopic vision.

Curved surfaces may appear light or shaded, depending on their slope. In general, they may be considered as acting like lenses, to cause convergence or divergence of light. A few simple experiments with oil drops or air bubbles in water, or with glass rods and tubes, will indicate the character of the shading exhibited by spherical or cylindrical objects (Fig. 133).

It should be borne in mind that the opacity of shadows is governed chiefly by the refractive indices of object and mounting medium, and that their width and sharpness depend principally upon the size, position, and curvature of the surface which causes them.

The third dimension, depth or thickness, is not apparent in the ordinary microscopic image, and the solid structure of the specimen is easily misinterpreted. On account of the relatively slight depth of focus of the microscope, objects are seen under distinctly unnatural conditions as compared with direct visual observation, and may seem thicker than they really are. The small portion which is in focus at any one time gives an "**optical section**" which may be thought of as analogous to a thin section prepared by mechanical methods, or a "contour line" on the three-dimensional object. Simple structures, such as the geometrical forms of crystals, may be worked out from a series of observations made at different positions of focus. If necessary, drawings may be made to record each appearance, and to aid in following its gradations into the others at different levels (Fig. 79). Objectives of little depth of focus are preferable for such work. The experienced microscopist continually manipulates the fine adjustment as he makes an examination, in order to reveal a series of optical sections in succession and to follow the transitions of appearance; by this means he may obtain a very adequate impression of the structure from its "top views" only. Any shaded surfaces may be studied to ascertain whether they slope inward or outward.[2]

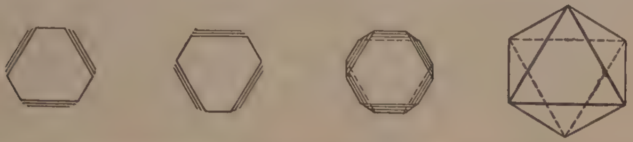

FIG. 79. "Optical Sections" at Top and Bottom Levels of a Flattened Octahedral Crystal.

Focusing methods are of particular value in the study of crystals by transmitted light, and in the examination of etching pits or elevated areas in metals. It is sometimes very difficult to decide whether a depression or a projection is present, without such procedure.

In applying focusing methods to studies of inclined surfaces or other three-dimensional features, strictly axial light should be employed, or the swaying of the image may render interpretation difficult. Even with axial or symmetrically convergent illumination, the appearance of the details as they go out of focus may simulate structures which do not exist. There is also some risk of mistaking a spurious, out-of-focus image for a perfectly focused one, especially if objectives of low aperture are employed (Fig. 80).

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[2] The slope may be measured quantitatively. See page 408.

Oblique illumination is invaluable in the interpretation of three-dimensional structures, since a very realistic shading and impression of "relief" are produced (Fig. 133). It is often useful in photomicrography as an aid in imparting a natural appearance to specimens which would otherwise seem unduly flat and lacking in depth.

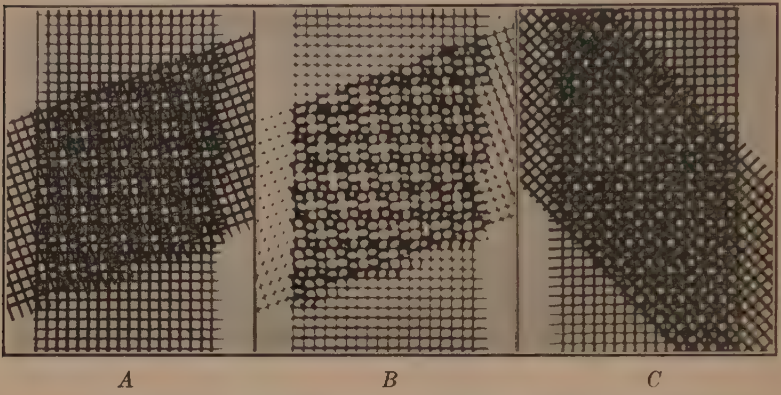

FIG. 80. Spurious Images from Superposed Structures.
A — crossed screens, in focus.
B — *A*, out of focus.
C — crossing at another angle.

Spiral structures, such as occur in certain vegetable textile and paper fibers, are not reversed under the microscope, as far as their right- or left-handed character is concerned. Diffraction patterns and haloes at the boundaries of fine structures may be misinterpreted as indicating surface films or coatings, if care is not taken to study the specimen with different types of illumination.

The microscopist should constantly strive to acquire skill in recognizing the appearance of simpler structural elements, since almost any complex image may be analyzed and studied piecemeal by the above methods. Simple known structures, examined under a variety of conditions of illumination and at different magnifications, are particularly helpful.

The binocular microscope, if it yields true stereoscopic vision, is very convenient for the observation of three-dimensional structure, and may give a very realistically "plastic" image, in which differences of elevation are strikingly evident, even without change in the focus of the microscope.

Serial sections have already been mentioned as valuable in reconstructing the form of complicated objects. Their use in the preparation of three-dimensional models is described in detail by Gage.[3] Sections in different directions through the specimen are very useful in revealing its true form.

It is highly desirable to be able to study an object in different positions, in order to observe different " views " of it. If the " plan " and the " elevation " or " cross-section " can be observed, interpretation is greatly facilitated. In the case of objects which are more or less equidimensional, it may be possible to orient them under the microscope so as to reveal different aspects of their structure, but this is rarely possible in the case of elongated or flattened forms, such as needle or plate crystals, or textile and paper fibers. A careful drawing of the " plan " of a crystal or other simple solid may be projected to give a fairly useful indication of its " elevation " in longitudinal or transverse section. (Figs. 32, 118, 122.)

It sometimes happens that objects distributed at random throughout a preparation may lie in all possible orientations, if their form permits, and from a study of several different ones some conclusion may be drawn as to the typical shape; this is particularly useful in the study of crystals or similar geometrical objects. Occasionally a viscous mounting medium may be used to support the specimens in various positions, by stirring it about with a fine needle. A simple device for orientation, often perfectly satisfactory, consists in cementing the object to the point of a needle or a fine glass rod, the other end of which may be inserted in "Plasticene." The specimen can be revolved or moved in various directions, and secured in place by gentle pressure upon the "Plasticene." Solid angles of tiny crystals may be computed from measurements of the plane angles of different faces. The specimen may be immersed in a shallow cell filled with liquid, to give greater transparency. Another method is to insert the object in a tiny glass capillary, which is filled with, and immersed in, liquid of the same refractive index as the glass. Rotation may be effected by hand, or by a micrometer drum, as in the Greenough capillary rotator of Zeiss. Klein's orientating apparatus (Fuess) is similar in principle.

The surface character of an object may sometimes be deter-

[3] *The Microscope* (1925), pp. 410–23.

mined by taking an impression of it in transparent material; this may be sectioned, or examined by various sorts of illumination. The method is valuable as a means of eliminating spurious effects in the image of the original, which are not due to surface structures and which might invalidate the direct image.[4] Syrupy collodion or "Duco Household Cement" may be applied, allowed to harden, and stripped off; it gives a very exact replica of the surface on which it was cast.

The behavior of the specimen when disintegrated mechanically or chemically may give an indication of structures which are not visible under ordinary conditions. Swelling to incipient gelatinization, maceration[5] to separate tissue elements, or crushing, tearing, bending, and breaking in tension may reveal details which are not otherwise indicated. Such methods are particularly applicable to textile fibers, woody tissue, food materials, and similar substances of organic origin, which may thus be more or less disintegrated into subcellular structures.[6]

OBSERVATIONS OF COLOR

Color phenomena and colored objects are of very great importance in microscopical investigations. Most of the factors which affect the observation of color under the microscope function in macroscopic observations also, and study of them is a valuable aid to an understanding of the methods, principles, and terminology which chemists encounter in the description of colored materials, whether microscopical or not.[7]

Subjective factors affect the observation of color very materially. Few persons are color-blind, but many are lacking in training in discrimination or in ability to describe what they see. Temporary color-blindness may be induced by prolonged exposure of the eye to strong light of a given color; an eye fatigued by red light sees all objects unduly green for a time. Similarly, daylight illumination of microscopic objects makes them appear bluish, after one has been working with yellowish artificial light.

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[4] Foster: *Trans. Amer. Soc. Steel. Treat.* **5**, 413 (1924).
Mason: *Jour. Phys. Chem.* **31**, 1871 (1927).

[5] For reagents, see Lee: *op. cit.*, p. 250.

[6] See also the discussion of mechanical properties on page 196.

[7] A general discussion of color phenomena of various types is given in the series of papers by Bancroft on The Colors of Colloids: *Jour. Phys. Chem.* **22–23** (1918–19).

Contrast effects from juxtaposed objects of different colors may vitiate the simplest observations; a colorless object in the midst of a field of red will appear greenish. The importance of these facts in the study of stained preparations is obvious, particularly if fine color discriminations have to be made.

Illumination by colored light has already been discussed as a means of increasing or decreasing contrast and transparency of colored objects (page 101). It is not generally realized that slight variations in illumination, such as those existing between daylight and artificial light, may cause entirely false observations of color.[8] Certain blue dyestuffs appear red by slightly yellowish light,[9] and many colored substances exhibit this phenomenon to a less degree.

Chromatic aberration in the microscope may lead the inexperienced observer to ascribe the faint green or pink residual color to the object itself, but one soon learns to make allowances for this. In highly critical observations of very fine structures, faintly colored, it is better to use apochromatic objectives.

Spectroscopic Eyepieces (Microspectroscopes) are employed for the qualitative and quantitative study of materials yielding absorption spectra, but are inconvenient and impracticable for bright-line spectra.

Dispersion is usually obtained by an Amici direct vision prism of either three or five units mounted above a negative eyepiece of special construction, in which the slit of the spectroscope is placed in the plane of the diaphragm of the eyepiece. Usually a small reflecting prism is provided which, when swung into position below the slit, cuts off approximately half the width of the spectrum from the object under the microscope and permits placing in juxtaposition with this spectrum the spectrum of a standard of known nature and composition.

For recording the position of absorption bands or the extent of absorption at the ends of the spectra an arbitrary scale or a scale reading directly in Angström units is generally provided. Instruments of this type are not suitable for chemical investigations. A better class have attached to them a micrometric device of considerable accuracy (Figs. 81 and 83).

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[8] Wood: *Physical Optics* (1919), p. 439.

[9] The well-known properties of the gem Alexandrite, which is green in daylight and red by lamplight, are of similar nature.

A typical example of one of the older and more commonly used spectroscopic eyepieces is shown in Figs. 81 and 82.[10] It consists of a cell or chamber *K* in which are housed the slit *s*, the comparing prism *p*, a movable diaphragm *d*, and in the lower opening the field lens *f* of the ocular. A small opening *O* in the side of *K* permits light, reflected by the mirror *m*, to enter the prism *p* and thus yield a spectrum in juxtaposition to that obtained from the object under the microscope. The solution or transparent solid used for comparison is held before the opening *O* by means of the clamps *CC*. The knob *P* serves to swing the comparing prism *p* beneath the slit or out to one side. *T* attached to a right- and left-threaded spindle serves to widen or narrow the slit *s*. Attached to the upper part of *K* is the remainder of the eyepiece with its eye lens *e* vertically movable by rack-and-pinion through the milled head *F*. Fitting above *e* is a tube *A* carrying an Amici prism *R*,

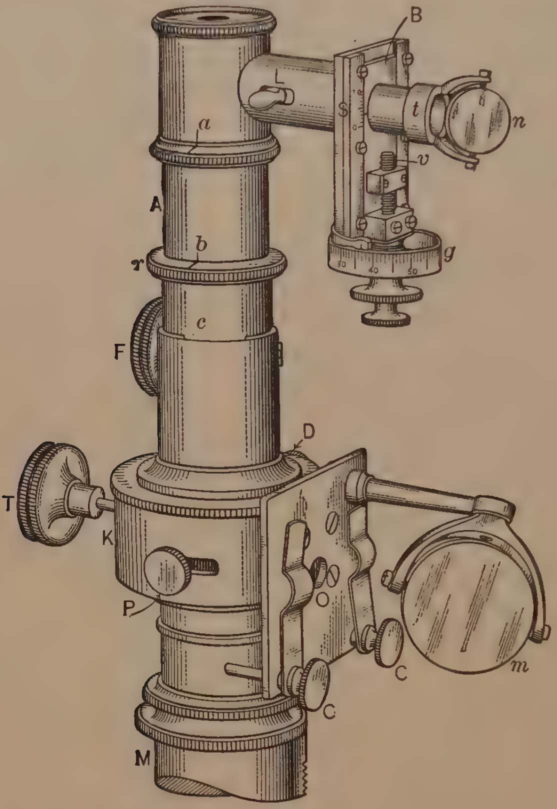

FIG. 81. Microspectroscope. (W. & H. Seibert.)

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[10] Made by W. & H. Seibert, Wetzlar, Germany

consisting of three prisms of crown glass ($n_D = 1.534$) alternating with two prisms of flint glass ($n_D = 1.587$).

Since the total deviation of a ray of light entering a series of prisms is equivalent to the sum of the deviations which would be imparted to it by each unit in turn, it follows from the alternate arrangement of the glass prisms, three low and two high, that the deviation of the system will be the difference between the deviations produced by the crown and flint prisms. The net result is that for rays of medium wavelength (yellow-green) the path of the emerging rays lies substantially in the same line as that of the rays entering the system, hence it is usual to term such a prism system a *direct vision* prism. The dispersive power of such a system is equivalent to that which would be produced by the prisms of flint glass alone. In the diagram (Fig. 82) the total dispersion indicated is therefore not theoretically correct.

The measuring service of the Seibert microspectroscope fits above the tube A. It consists of a diaphragm with a very tiny triangular opening I mounted in the sliding plate B and illuminated by the mirror n; an image of this opening is projected by the lens l as a tiny bright white triangle upon the inclined surface of the prism R and is then reflected to the eye at i. The knob L serves to slide the lens l and thus focus the image of the triangular opening. The plate in which the diaphragm is mounted can be displaced vertically by means of a micrometer screw; the amount of displacement is indicated upon the scale S and by the graduations upon the drum g; one complete rotation of the drum (100 divisions) is equivalent to one division of the scale S.

To facilitate the illumination of the diaphragm opening I, the mirror n is attached to a rotating collar t.

The position of a line in the spectrum is ascertained by bringing the triangle image to such a position that the line bisects the vertical angle. The scale and drum divisions are then read and recorded. The equivalent of this reading in wavelengths is obtained from the calibration of the instrument by the method given below.

Should the object, whose absorption spectrum is to be studied, be so small that its image fails to fill completely the length of the slit, the slit must be shortened until the object *completely* fills it and there will be no light reaching the eye which does not first pass through the object. This is accomplished by pushing the comparing prism into place, thus cutting the spectrum in half. At the same time the mirror m is turned aside so that no light enters O. Should the image of the object still fail to fill the length of the slit, the diaphragm d is moved toward the center by turning the head D until the slit length is reduced to proper dimensions.

Before a spectroscopic eyepiece can yield scale readings convertible into wavelengths, it must be calibrated. In the case of instruments of the type shown in Fig. 81 this will necessitate placing upon its tubes certain reference or indicator marks. The instrument is removed from the microscope tube M, pointed toward the sky and the slit narrowed. The spectrum should appear as a long rectangular band of colored light crossed by many fine black lines at right angles (Fraunhofer lines) to its length. Should these lines appear inclined, the tube *A* must be turned slightly until they are made normal to the spectrum length. Having thus carefully adjusted the prism to the proper position with reference to the slit, make

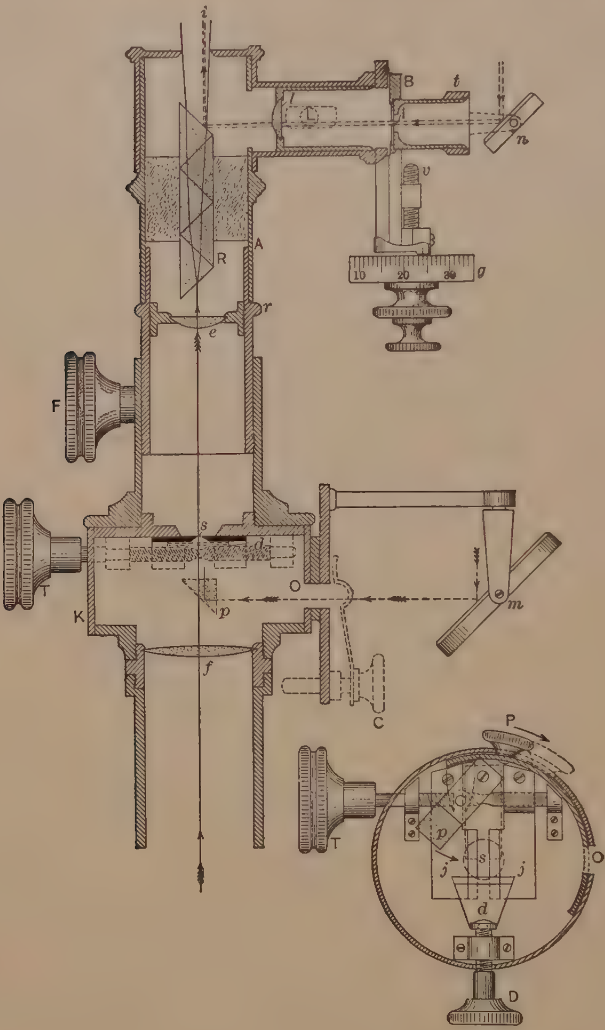

FIG. 82. Microspectroscope. (W. & H. Seibert.)

the reference marks *b* upon *A* and upon *r* in order to fix this position. Now carefully focus the spectrum by means of *F*, using the narrowest slit possible until the Fraunhofer lines appear sharpest. This should be done on a bright sunny day. Scratch the mark *c* to indicate this position. Turn *t* and tip the mirror *n* so as to reflect light into the tube and move *L* until a bright sharp white triangle is seen when looking into the eyepiece. Carefully turn the cap carrying the measuring device until the apex of the bright triangle takes a position just a trifle above the center of the spectrum band. This position is easily ascertained by pushing the comparing prism in place beneath the slit; half the spectrum will now disappear. The most convenient position for the bright spot of light is when the base of the triangle falls just below the dividing line. Make the marks indicated at *a* so as to fix this position. The instrument is now ready for calibration. It can be taken apart at any time and the parts replaced so as not to alter the values of the scale divisions. After calibration, if at any future time wavelength measurements are required the instrument is first set so that all the reference marks take the same positions as when the spectroscope was first adjusted.

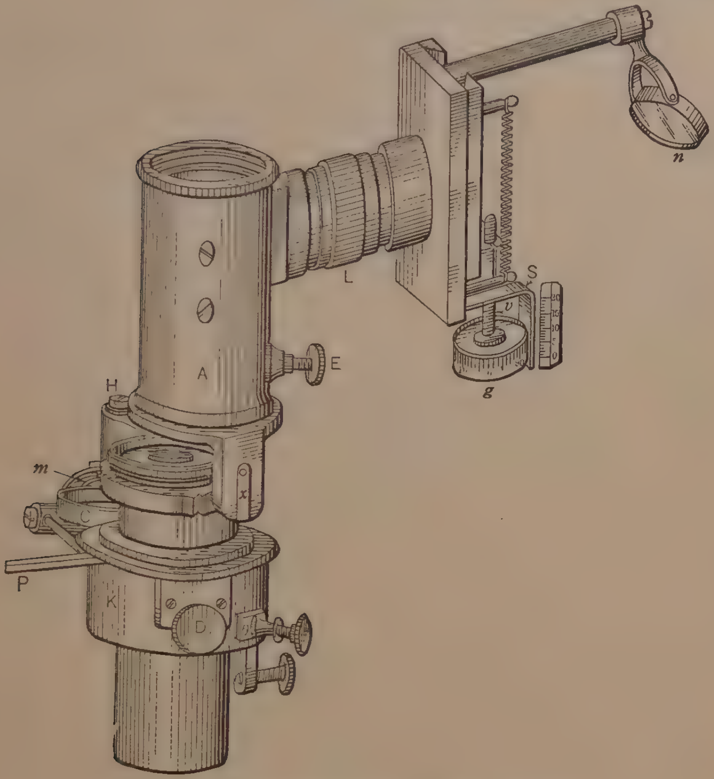

FIG. 83. Microspectroscope. (Zeiss, modified.)

Measurements of line or band positions are made by bringing the bright white triangle to such a position that the line or the edge of the band bisects the acute angle of the triangle. The scale S and drum g are then read and recorded. S reads from 0 to 10, g in hundredths of S. For example, in the instrument illustrated: Fraunhofer $c = 0.42$, $D = 1.41$, $G = 7.11$, etc.

A more convenient and practical type of spectroscopic eyepiece is shown in Figs. 83 and 84.[11]

From the figures together with the description given above, the main features of the Zeiss instrument will be readily understood since similar component parts are lettered alike in all four figures. There are, however, several features that render the Zeiss spectroscope more convenient: a milled headed screw E tips the prism R and thus provides a means of adjustment with reference to the scale of the spectroscope; the entire prism mounting may be swung aside[12] on the hinge H, a click spring x holds the prism in alignment; the slit is attached to a sliding plate w and may be displaced by pulling on T thus bringing into the axis of the microscope a circular opening U which allows an unobstructed view of the field. When the object has been centered and studied, the slit is pushed back into the axis of the microscope

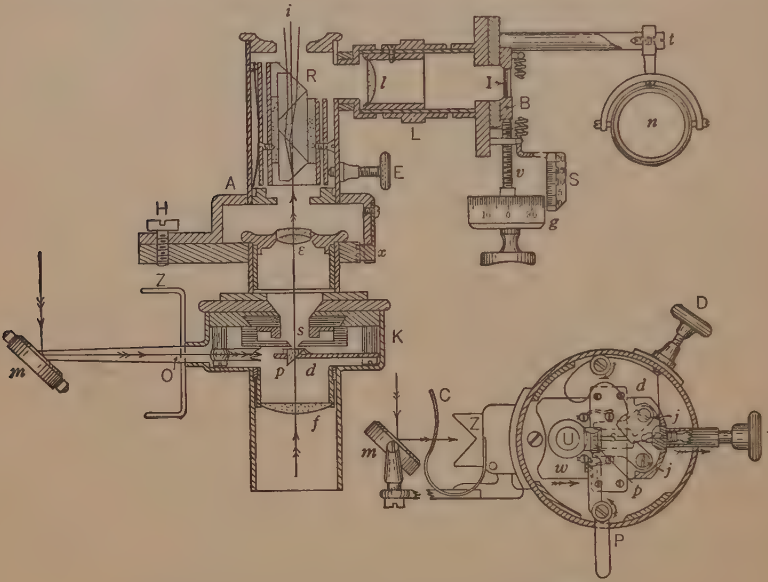

FIG. 84. Microspectroscope. (Zeiss, modified.)

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[11] Manufactured by Carl Zeiss, Jena, but improved by substituting for its fixed Angström scale a Krüss micrometric measuring device, analogous to that used on the Seibert instrument (but instead of a bright triangle a bright white line is made to travel the length of the spectrum).

[12] In the new model of E. Leitz this valuable feature has also been adopted.

and the prism *p* and the diaphragm *d* are adjusted so as properly to restrict the area illuminated to that particular portion of the object whose absorption spectrum is under investigation. The prism *R* is then swung in place, the slit adjusted by means of the micrometer screw *T* and the necessary spectroscopic observations made and recorded. The screw *W* serves to clamp the instrument securely in place upon the draw-tube of the microscope.

In calibrating scales by means of the Fraunhofer lines direct sunlight should be thrown into the instrument by means of the microscope mirror. For bright lines, hold the instrument clamped securely in place on a suitable clamp stand and direct it toward a Bunsen burner flame into which the metallic salts are to be introduced. The following lines will be found convenient for the calibration:

Line	Corresponding wavelength in Angström units	Line	Corresponding wavelength in Angström units
A	7600	F	4681
K_α	7682	Sr_β	4607
a	7201	Cs_α	4555
B	6870	Cs_β	4593
Li_α	6708	d	4383
C	6563	G	4308
Na (D)	5893	g	4226
Ba_α	5535	Rb_β	4215
Tl	5350	Rb_α	4202
E	5270	h	4103
b_1	5183	H_1	3968
b_2	5173		

When only approximate results in terms of wavelengths are needed, a very convenient device consists in plotting the curve for the spectroscope upon coördinate paper, using wavelengths as ordinates and scale divisions as abscissas. Such a calibration curve is shown in Fig. 85, the black dots indicating the measurements actually made.

For the study of the absorption bands of liquids under the microspectroscope, the most convenient cells will be found to be tubes of different size bores and of different lengths mounted as described on page 190, or a cell of the type devised by Andrews. This consists of a glass tube about 2 cm. in diameter and of any convenient length (Fig. 86) cemented upon an object slide with DeKhotinsky cement. The observation tube about 5 mm. in diameter has its lower end closed by a piece of plane glass cemented to it; it may be raised or lowered in its rubber support (section of a rubber stopper) for the purpose of increasing or decreasing the thickness of the liquid layer.

The position of maximum intensity of an absorption band should always be determined by observing the situation of the vanishing point of the band after repeated dilutions.

It should be borne in mind that the position of a band may be changed greatly through increased or diminished dissociation, and that the absorption bands given by a crystal may be quite different from those given by the same material in solution and furthermore that the absorption spectra are usually different in different directions through the crystal.[13]

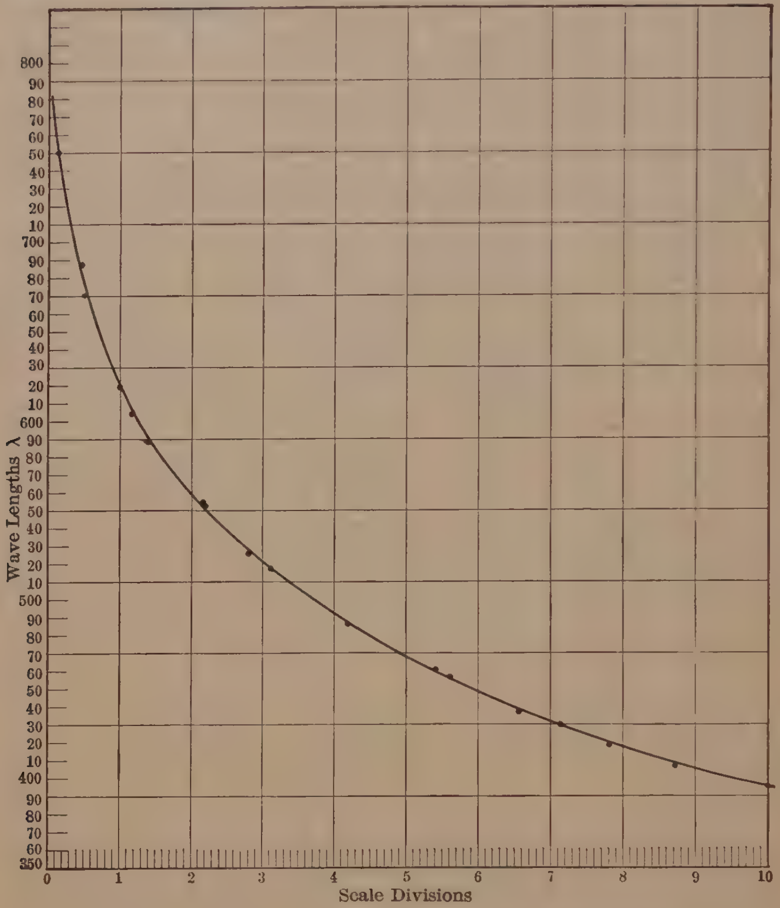

FIG. 85. Calibration Curve of a Seibert Microspectroscope.

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[13] For further discussion of spectroscopic eyepieces and their applications see:
MacMunn: *The Spectroscope in Medicine* (London, 1880).
Engelmann: *Zeits. wiss. Mikros.* **5**, 289 (1888).
Brasch: *Über Verwendbarkeit der Spektroskopie z. Unterscheidung der Farbenreaktionen der Gifte* (Dorpat, 1890).
Blyth: *Poisons; Their Effects and Detection* (London, 1895), p. 54.
Keeley: *Proc. Acad. Nat. Sci. Phila.*, **63**, 106–116 (1911).
Wychgram: *Zeits. wiss. Mikros.* **29**, 339 (1912).

Records of color are most easily made with reference to a chart of standard hues, tints, and shades,[14] using a comparison eyepiece if desired.[15] The drawing camera may be used to view the object and the color standard simultaneously, or the image may be projected in juxtaposition with the standard.[16] Comparisons of color with standard specimens may be effected by means of an ordinary comparison microscope; this may be applied to opaque mineral grains,[17] dyed fibers, color reactions, and other microscopic materials. The color of the standard may be projected in the field of the microscope by means of the condenser (page 43); determinations of hydrogen-ion concentration by indicators may be made in this way[18] on single cells, organs, and tissues.

Polarization colors are best described and compared with color charts of Newton's series, or with compensators of doubly refracting material. (See page 283.)

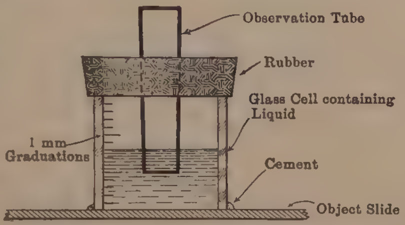

FIG. 86. Andrews' Cell for Absorption Spectra.

Colorimetry may be carried out on a microscopic scale by methods analogous to those employed in dealing with larger quantities of material.[19] A comparison microscope or a comparison eyepiece is used (page 68) and the samples, of known

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Wherry: *Microspectroscope in Mineralogy.* Smithsonian Misc. Coll. **65** (1915), No. 5; *Amer. Mineral*, **14**, 299 (1929).

Gage: *The Microscope* (1925), p. 246.

Krause: *Enzyclopädie der mikroskopischen Technik* (1926), Bd. II, p. 1522.

Metzner: *Das Mikroskop* (1928), p. 216.

[14] Mulliken: *A Method for the Identification of Pure Organic Compounds.* Vol. I (1904), pp. 231–4.

Munsell Color Chart. Prepared by the Munsell Color Co., Baltimore, Md.

[15] The comparison microscope of Bausch & Lomb (unfortunately mounted on a poorly designed stand) is provided with slots for the insertion of Lovibond tintometer glasses, for matching and recording colors.

[16] Davis and MacLaughlin: *Science* **67**, 71 (1928).

[17] Talmage: *Econ. Geol.* **20**, 168 (1925).

[18] Patin: *Nature* **111**, 81 (1923).

[19] Yoe: *Photometric Chemical Analysis.* Vol. I, Colorimetry (John Wiley & Sons, New York, 1928).

and unknown concentrations, are viewed simultaneously in the divided field. Liquids may be contained in cells like that shown in Fig. 86; a better type of cell, requiring only a drop or two of liquid, may be made from a short length of glass tubing, 1 to 3 mm. in inside diameter, the ends of which have been ground true. The tubing is held upright on a microscope slide, either by cement or better by supporting it in a closely fitting perforated piece of rubber or cork which has been cemented to the slide; the latter arrangement permits easy cleaning. Cells of various heights should be available, and those used in comparisons should be of equal diameter. In order to prevent internal reflections which might render the results inaccurate, stray light is excluded by blackening the end of the tube; the outside of the cell should also be blackened or covered with a sleeve of black paper.

A solution containing a known concentration of the colored substance to be determined is placed in one cell; the other contains the solution of unknown concentration. The transmission colors of these two solutions are compared by means of a comparison microscope or comparison eyepiece, the depth of the liquid being adjusted until the color in the two halves of the field is of the same intensity. The depths of the columns of liquid are then carefully measured, preferably by means of a pair of dividers, a simple magnifier, and a finely divided steel scale. Other factors being equal, the relative concentrations are inversely proportional to the relative thicknesses of the two solutions. For accurate results the concentrations of the unknown and the standard solution should not be very far apart. A sensitive assay or micro-balance must be employed for weighing out the materials in making up the solutions.

It is absolutely essential that the fields of the two microscopes shall be illuminated *with equal intensity*. The cells are filled with distilled water, centered in the fields, and the instruments focused on their upper level. By tilting the microscope mirrors the two halves of the field may be made equally bright. A light source of considerable area is desirable; a square of ground glass or a sheet of pure white paper in front of a window is satisfactory.[20]

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[20] See also: Emich and Donau: *Monatshefte* **28**, 826 (1907). Donau: *ibid.* **36**, 381 (1915). Emich: *ibid.* **36**, 407–440 (1915). Donau: *Die Arbeitsmethoden der Mikrochemie* (Stuttgart, 1913). Emich: *Lehrbuch der Mikrochemie* (Munich, 1926), p. 61.

Nephelometry or measurement of turbidity of suspensions or emulsions may be carried out by an analogous procedure.[21]

Body or transmission colors are exhibited when light passes through the object to the eye (page 77). They are best observed by dark field illumination in the case of faintly colored, finely divided material, since the excess of white light in the field is thus eliminated. Fluorescence or surface color, if present, may also be manifest under these conditions. Even highly opaque substances (excepting some metals) will show transmission colors in thin flakes or at the edges of splinters. The body color of anisotropic materials may vary with the direction in which the light travels through the specimen, and for a given direction, with the plane of vibration of the light (pleochroism, page 286). Thickness may affect the apparent body color of certain substances very markedly; dyestuffs and other highly colored materials, when in thin layers, may be of entirely different hues than when in thick layers.[22] Observations of the color of solutions or dyed fibers may be affected by this, since variation in concentration of the coloring material can have a similar effect on the hue, entirely apart from any dissociation or reaction with the solvent.

Body colors are less marked the more finely divided the specimen, and some nearly opaque precipitates may be relatively pale if composed of very fine particles. Digestion of the precipitate, with increase in the size of the particles, may cause a darker shade to be developed. Observations of the body color of fine-grained materials should always be made with the naked eye as well as with the microscope, for substances which are not very strongly absorbing may appear almost colorless in thin layers, though distinctly colored in mass. It is advisable to eliminate the effect of refraction by mounting the colored substance in a medium of its own refractive index, if possible. This will give greater transparency, and will render comparisons (such as the strength of dyeing on fibers) more reliable. Phenomena of structural color will be nullified by this procedure.

Surface or reflection colors are due to selective reflection of part of the light which falls upon the surface of a substance; they are observed only in strongly absorbing materials, and are approximately complementary to the body color. The reflection is specular, so that the illuminating beam and the reflecting surface must be at the proper angles to send light to the microscope. To observe the surface color unmixed with the body color, vertical or inclined illumination from above the stage is best. Care should be taken to note the light which is reflected from the upper surface of the object, rather than that which has entered and been internally reflected. Surface color is sometimes exhibited with dark field illumination, especially in the case of finely divided, highly opaque material. Substances possessing the power of specular selective reflection are usually so strongly absorbing that they tend to appear dark when finely divided, unless the particles are so thin as to be transparent. The study of a few typical examples of materials showing surface color is worth while for the beginner; for instance: fuchsin, or aniline blue, crystals or a film; indigo, sublimed crystals; potassium permanganate; copper powder; lead tree.

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[21] See also Conklin: *Jour. Optical Soc. Amer.* **10**, 573 (1925).

[22] This phenomenon of *dichromatism* is discussed by Wood: *Physical Optics.*

The hue which is selectively reflected may be different for different faces of anisotropic crystals. It usually varies markedly with the plane of vibration of light, if a polarizer or analyzer is used. Slight differences of hue may be noticed with changing angle of reflection, especially if polarized light is employed. Surface color is sometimes altered markedly by immersion in media of different refractive indices.

Fluorescence, and the **colors of colloidal suspensions**, together with the means of recognizing each, are discussed on pages 79 and 231.

Structural colors are important in microscopy because they originate in fine structures and frequently occur in objects which are studied by the microscope. They are also most readily recognized and identified by microscopic methods.[23] The various types of structural colors are due, not to any body or surface color of the material but to the conformation of its surfaces; perfectly colorless substances may give rise to brilliant colors, if the proper structure is present. Structural colors are sometimes superposed upon body colors, and may complicate observation of them.

Since all structural colors depend on optical phenomena of reflection, refraction, and diffraction, it is evident that, if these effects can be eliminated, the color will be destroyed. In the case of any structure in which one of the phases is a liquid or gas, it is possible to substitute for it a permeating liquid of such refractive index that the system becomes *optically homogeneous*, and the color vanishes. Removal of this liquid, and replacement of it by another fluid of different refractive index, will generally restore the original color.

Structural colors are usually dependent upon the size or shape of the fine structure which causes them, and if this is altered by mechanical or chemical means, such as crushing, swelling, compression solution, or growth, the color either changes or is entirely lost.

Luster and opacity of colorless materials depend very largely on the character of their surfaces; and on their power of scattering light instead of reflecting it more or less specularly. The luster of textile fibers,[24] the opacity of papers,[25] pigments,[26] and frosted glass,[27] and the brightness of polished surfaces, all depend on microscopic structural features. Transparency and light-scattering ability have a pronounced effect upon the apparent tint of samples of colored substances such as dyed fibers and papers, colored pigments, wood, leather, and other materials, which may be actually identical as regards their true body color. In nearly all studies of color, the influence of the surface texture of the specimen has to be taken into account.

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[23] Different kinds of structural colors and criteria for their identification are discussed by Mason: *Jour. Phys. Chem.* **27**, 201, 401 (1923); **28**, 1233 (1924); **30**, 382 (1926); **31**, 321, 1856 (1927).

Oriented luster results from a more or less systematic and parallel arrangement of minute reflecting surfaces, such as microscopic etching pits or scratches in metals, fissures or inclusions in minerals, and fibrous or flaky suspended matter in plastics and enamels. The material exhibits good reflecting power in certain directions and much less in others, although there is no apparent external difference in the angles of incidence and reflection. The explanation of such phenomena, and their elimination when they are objectionable, depends on a microscopic investigation of the actual character and orientation of the reflecting surfaces within the specimen. Conversely, the directions of maximum reflection may be studied as a means of investigating the orientation of the minute reflecting surfaces.[28]

White is a structural effect in all cases, and is due to strong reflecting and refracting properties. Colorless, transparent objects with many fine, unordered surfaces appear white by every sort of illumination except transmitted light; with it, their excellent light-scattering power renders them opaque, so they appear black against a bright field. Apparently opaque precipitates obtained in microchemical reactions should always be examined by inclined illumination from above the stage, for this reason; if faintly tinted, their color will be made visible under these conditions when otherwise it might be missed.

Turbidity or cloudiness is closely related to whiteness, in that the light is scattered by fine suspended particles. Dark field illumination is useful in comparing samples of turbid materials, and polarized light may be used to advantage if the suspended particles are doubly refractive.

Black may be due to the opacity of a white material by transmitted light, to the strong absorption of light by a truly opaque substance (such as carbon), or to a fine state of subdivision in the case of a specularly reflecting opaque material. Pulverulent metals (such as lead or silver "trees," page 340) look dark by reflected light, except where some tiny crystal face is in position to reflect.

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[24] Herzog: *Kunststoffe*, **6**, 153, 166 (1916) — *Mikroskopische Untersuchung der Seide und der Kunstseide* (Berlin, 1924), pp. 71–81.

[25] Thiriet: *Chim. et Ind.* Spl. No. Sept. **1925**, p. 488.

[26] Stutz and Pfund: *Ind. Eng. Chem.* **19**, 51 (1927).
Kühn: *Farben Ztg.* **31**, 1131 (1926).
Hebler: *idem.* pp. 1739, 1906.
Gademann: *idem.* p. 1850.

[27] Pipkin: *Ind. Eng. Chem.* **18**, 774 (1926).
Spencer and Ott: *Jour. Amer. Ceram. Soc.* **10**, 402 (1927).
Masuda: *Jour. Jap. Ceram. Asso.*, **36**, 183 (1928).

[28] Czochralski: *Zeits. anorg. Chem.* **144**, 131 (1925).
Tammann and Meyer: *Zeits. Metallkunde* **18**, 339 (1926).
Tammann and Botschwar: *Zeits. anorg. Chem.* **175**, 121 (1928).

The Christiansen effect[29] is due to differences in the dispersive power of substances which have almost the same refractive index. It is manifest when a material is immersed in a liquid of refractive index the same as its own for certain colors, but distinctly different for others. Under these circumstances light of the color for which the refractive indices are identical will be transmitted, and the other colors will be deviated by refraction. As a consequence, the particle appears faintly colored by transmitted light. This phenomenon is frequently noticed in determining the refractive index of crystals by the immersion method; it is the cause of the apparent pale pink color of precipitated crystals of sodium fluosilicate, and is probably the explanation of the more brilliant colors shown by the liquid-crystalline phase of cholesteryl compounds.

Colors due to the Christiansen effect are very susceptible to changes of temperature, which alter the relative refractive indices of the system and thereby change the hues of the transmitted and scattered light (but not their complementary relationship). The colors are most marked with moderately finely divided particles, but are absent if the dimensions are less than several microns. At least one of the two phases must have rather strong dispersive power. Admixtures to the liquid phase which change its dispersion alter or destroy the colors. Stirring or other disarrangement of the material has no effect upon them.

Tyndall blue[30] is exhibited by fine particles of relatively transparent material which are approximately 0.3 μ in diameter. Such particles are large enough so that they scatter light of short wavelength, but they are too small to deviate the longer waves and these are transmitted. As a result, the transmission color is a turbid orange and the scattered light is a turbid blue. Tyndall blues are exhibited by finely divided precipitates such as silver chloride or sulphur, by very fine paint pigments such as certain grades of zinc oxide, and by many other fine structures in microscopic objects. The blue color is best observed by dark field, orthogonal, or inclined illumination. The brownish color which is frequently observed in aggregates of very small particles, such as spherulites and sheaves of fat crystals, is due to the same causes as Tyndall blue, of which it is actually the transmission color.

Tyndall blue may be identified by the characteristic color of the scattered and of the transmitted light, and by direct observation of the fine structures by an objective of high resolving power. The scattered light is polarized, as in the case of other colloidal suspensions (page 230). If the system can be rendered optically homogeneous, by completely surrounding the fine structures by a medium of their own refractive index, the color vanishes.

Transmitted blue is primarily a microscopical phenomenon. It is observed with structures which are colorless and relatively transparent, of good contrast, and of dimensions larger than about 1 μ; the numerical aperture of the objective is also important. The structures or particles need not be in any orderly arrangement, but must be fairly uniform in size, and just fine enough so that only the blue light diffracted by them is included within the angular cone of the objective; the illumination cone must be rather narrow. Transmitted blues of this type are observed in fine precipitates, sublimates, and some specimens of pearlite in steel.

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[29] Wood: *op. cit.*, p. 110. Holmes and Cameron: *Jour. Amer. Chem. Soc.* **44**, 71 (1922).

[30] Wood: *op. cit.*, p. 624.

The recognition of transmitted blue is simple; it is not visible by dark field or inclined illumination, and its color is lost if an objective of higher aperture is used or if a wide illuminating cone is employed. Rendering the system optically homogeneous of course destroys the visibility as well as the color of the fine structure.

Grating colors are produced when any system of more or less regular and parallel fine structures is illuminated by unidirectional light crosswise of the structures. The effect is that of a diffraction grating, and the light emitted at any angle depends on the dimensions of the structure and the direction of the illuminating beam; the whole range of the spectrum is possible. Colors of this type are rarely encountered in chemical microscopy, because sufficiently perfect periodic structures do not generally occur except from living organisms. Diatomaceous earth, such as is used for filtering and insulation, may contain some diatoms which exhibit grating colors with dark field illumination. Pearlite in steel owes its name to this color phenomenon, which is best observed with oblique vertical illumination.

Diffraction colors may be identified by a study of the structure from which they originate. From the laws of diffraction, together with a knowledge of the arrangement of the fine details of the object and the direction of the light, it is generally possible to prove the nature of the color. Very marked changes in hue result from a small change in the angle of illumination. Furthermore such colors disappear in uniform light and are not visible with a wide-angled illuminating cone and an objective of large aperture. They are only observable if the illuminating beam is in the proper azimuth, and are best seen against a dark background. Rendering the system optically homogeneous, by permeation with a medium of the proper refractive index, destroys the color and proves its structural origin. Illumination with strictly unidirectional light (transmitted or reflected) may render the diffraction spectra visible at the back aperture of the objective.

Thin film colors[31] are due to interference between light waves reflected from the two surfaces of a very thin film of relatively transparent material. Certain colors are destroyed and others are reinforced, the hue depending on the thickness of the film; the whole range of colors is comprised by Newton's series, and the brightest hues originate from films 0.5 μ to 1.5 μ thick. Thin film colors are exhibited by substances which crystallize in thin plates, such as lead iodide; by oxide films on metal specimens, especially surrounding pits or after heat tinting or tarnishing; and by films of silica or other material formed by the attack of chemicals on the glass of bottles or slides.

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[31] C. V. Boys: *Soap Bubbles* (1912). Wood: *op. cit.*, p. 160.

The actual existence of a thin film is presumptive evidence as to the origin of the color in any specimen. The characteristic hues and also the sequence exhibited with slight variations in thickness or in the angle of reflection or transmission are fully as distinctive. The specular nature of the reflection and the complementary relationship of reflection and transmission colors, together with the paleness and lack of saturation of the latter, are useful criteria. Thin film colors are best seen by reflected light against a dark background. They do not disappear in uniform light, but do so if the system is made optically homogeneous by means of a liquid having the same refractive index as the film. In order to avoid confusing thin film colors of plate-like crystals with body colors, the preparation should be shaded by the hand, to cut off reflected light from above the stage.

OBSERVATIONS OF MECHANICAL PROPERTIES

The interpretation of microscopic appearance in terms of the mechanical properties of a specimen may be indirect (as in estimating hardness or tensile strength of alloys from metallographic studies of their crystal character and phase composition), or the properties themselves may be determined under the microscope by more or less quantitative means. Only direct observations can be dealt with here.

Hardness may be measured by the standard Brinell method, which is commonly applied to metals and alloys. A hardened steel ball, 10 mm. in diameter, is pressed upon a smooth surface of the sample, under a standard load (3000 kg. for hard materials, and 500 kg. for soft materials). The "Brinell hardness number" is the ratio of the applied load to the area of the indentation produced. The area of the indentation may be calculated from measurements of its diameter or its depth, by means of a low power, large field microscope, or a simple magnifier with scale.

A drill operating under a definite load is the essential feature of Jaggar's "Microsclerometer."[32] The depth of the hole produced by a given number of revolutions of a diamond point is measured by means of the microscope. This method may be applied to a small area of the specimen.

Pressure by means of the point of a dissecting needle may be used to indicate hardness, plasticity, and elasticity. The behavior of the specimen may be followed under the microscope and valuable qualitative information may be obtained. Sharp or rounded points may be used, and the degree of pressure necessary to cause indentation or rupture of the specimen may be estimated by using a needle mounted in a holder with a fine coil spring. The holder is designed so that the spring is stretched as the pressure is increased, and its extension serves as a roughly quantitative measure of the force applied. The crushing strength of aggregates, such as primer pellets, may be tested conveniently in this way.

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[32] *Amer. Jour. Sci.* (4) **4**, 399 (1897).

More exact penetration methods have been devised, whereby a definite load is applied to a metal point or punch, the depth of the depression produced being measured microscopically.[33]

The most useful quantitative method of measuring hardness microscopically depends on scratching the surface of the specimen by a standard point under a definite load. The width of the scratch is measured, and from it the hardness is calculated. The particular advantage of this method lies in its applicability to very small objects, such as single grains in alloys or mineral aggregates.[34] A very ingenious apparatus called the "Micro-character" has been devised to permit accurate measurement of the scratch.[35] A tiny sapphire point, very carefully cut to the shape of a corner of a cube, is moved slowly and evenly across the surface of the specimen under a load of about three grams. By means of a centering device, any particular portion of the specimen to be tested may be selected under the microscope. The point plows deeper into soft grains than into hard ones, and the hardness is inversely proportional to the square of the width of the scratch.

A modification of the above method has been applied to the measurement of the average hardness of material consisting of an aggregate of fine grains, such as slate[36] and a similar procedure has been employed in measuring the hardness of varnish films.[37]

A critical review of the various methods of measuring hardness is given by Devries,[38] and an extensive bibliography is included in the Report of the A. S. T. M. Committee on Micro Hardness.[39]

It is not as yet possible to correlate perfectly the data obtained by various methods of determining hardness, but valuable comparative information is easily obtained on a wide variety of materials.

Wear resistance may be studied under the microscope by qualitative examination of the surface under various conditions and at various stages, and quantitative studies may be made of the amount of wear.[40] The action of various abrasives may be similarly investigated.

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[33] Such a method has been used in the study of the hardness of tooth enamel by Head: *Jour. Amer. Med. Ass.* **59**, 2118.

[34] A rough qualitative demonstration of the principle may be obtained by *lightly* stroking a very finely pointed scalpel or the sharp corner of a broken safety razor blade across the polished surface of an 80:20 *Cu-Sn* bronze, or a tin-base bearing metal. The width of the scratch varies markedly as it passes over phases of different hardnesses.

[35] Bierbaum: *Trans. Amer. Inst. Min. Met. Eng.* **69**, 972 (1923). Obtainable from the Spencer Lens Co. A less elaborate apparatus for similar measurements is described by Talmage: *Econ. Geol.* **20**, 531 (1925).

[36] Notvest and Booth: A. S. T. M. Comm. D–16 (1924). Private communication to the authors.

[37] Gardner and Parks: *Paint Mfrs. Ass. Circ.* **228**, 195 (1925).

[38] *Bur. Stds. Techn. Paper* **11**, (1912).

[39] *Proc. A. S. T. M.* **26**, Part I, 573 (1926).

[40] Anon: *Eng.* **101**, 149 (1916).

Jannin and Guillet: *Rev. de Mét.* **19**, 109, 117 (1922).

French: *Proc. A. S. T. M.* **27**, II, 212 (1927).

Ductility is not readily measurable directly by microscopic means, though it may be indicated by the behavior of the material under tension, compression, or bending.[41] Brittle materials break more or less cleanly, whereas ductile ones acquire a permanent deformation, and elastic substances spring back into shape when the stress is removed. The properties of textile fibers are indicated fairly well by their appearance when bent sharply, and by the ends of fractured fibers. Fibers which bend in smooth curves, such as wool, mohair, and silk, are elastic; those which bend sharply, buckle, fray, and split, such as flax and hemp, are not very elastic and loose their springiness after much mechanical treatment. Fibers which break off short when bent sharply, such as ramie, are brittle, although they may be strong under tension. The brittleness or friability of crystals and aggregates is often evidenced by their being easily crushed by pressure on the cover-glass. Cleavage in crystals may be recognized by the presence of definite faces and angles on broken fragments, and by parallel fissures where fracture is not complete.

Plasticity has been investigated microscopically in the case of suspensions of powdered materials in liquid,[42] by studying their flow through a capillary tube under definite pressure. A " microplastometer " apparatus is used for this purpose. From the pressure required to develop shearing forces in the sample, as indicated by the relative movement of particles in different layers, the yield value may be computed. The method is applicable to paints, clay slips, pastes, cements, starches, lubricants, and similar viscous or plastic materials.

Viscosity has also been measured by observing the velocity of a tiny particle of nickel moved through the specimen by means of a electromagnetic force of known magnitude.[43]

The modulus of elasticity and the elastic limit may be determined by similar means, and studies may be made on very minute amounts of materials, such as tissues and cell contents.

The presence and distribution of **strains** in various materials may be studied under the microscope with polarized light (page 303). Substances which are normally isotropic develop double refraction when subjected to stress, and this may be measured quantitatively by compensators (page 283), and tensile or compressive strains may be differentiated. Such procedure has been applied to glazes,[44] spun glass,[45] textile fibers,[46] and wood,[47] and is likely to prove of value in the examination of plastics.[48] The production of fissures and "gliding planes" in the specimen under strain may also be interpreted in terms of its submicroscopic structure.[49]

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[41] Ranke: *Zeits. wiss. Mikros.* **45**, 57 (1928).

[42] Green: *Proc. A. S. T. M.* **20**, II, 476 (1920); *Ind. Eng. Chem.* **15**, 122 (1923). Green and Haslam: *idem.* **17**, 726 (1925); **19**, 53 (1927).

[43] Freundlich and Seifriz: *Zeits. physik. Chem.* **104**, 233.
See also Metzner: *Das Mikroskop* (1928), pp. 412–4.

[44] Henry: *Jour. Amer. Ceram. Soc.* **8**, 117 (1925).

[45] Späte: *Glastech. Ber.* **4**, 121 (1926).

[46] Harrison: *Proc. Roy. Soc. Lond.* **94 A**, 460 (1917–8).

[47] Robinson: *Phil. Trans.* **210 B**, 49 (1921).

[48] Wächtler and Utesch: Liesegang's *Kolloidchemische Technologie* (Leipzig, 1927), pp. 957–9.

Observations of Motion. — Besides the reversal of motion in the non-erecting compound microscope, other illusions have to be guarded against. The velocity of currents in liquid or of Brownian movement may appear to be very great, on account of the magnification employed. It should of course be translated into terms of actual distance traveled in a given time, which may correspond to a very slow rate. Fortunately, diffusion phenomena, growth of crystals, cataphoresis, and other changes in form which involve movement of the image may be magnified in velocity many-fold, so that microscopic study is possible in a much shorter time than that with the naked eye.

The reversal of motion under the microscope is confusing when rotation is observed, as in the case of vortex action or diffusion currents in liquids. The apparent reversal of direction will not result in error if it is borne in mind that only lateral movement is reversed, and that vertical movement may be accurately followed by focusing.

Observations of Specific Gravity may be carried out by measuring the rate of fall of a particle of known dimensions through a liquid of known viscosity. For this work, the microscope is used in the horizontal position.[50] The method has been applied to the calculation of the composition of metal beads containing gold and other metals.[51] If the particle is large enough to be weighed on a microbalance, its specific gravity can be calculated from its volume. The various dimensions of a single crystal or other simple geometric form can be measured carefully under the microscope, and from them its volume can be computed. Soluble materials and rare substances can be studied in this way.[52]

Observations of Electrical Properties. — The determination of the sign and magnitude of the electrical charge on colloidal particles is discussed in Chapter VII. Single potentials at the surfaces of individual grains in alloys or mineral aggregates may be determined by means of a micro-electrode in the form of a capillary containing an electrolyte, which is brought into contact with the grain under the microscope.[53] Unpolarizable electrodes have been devised for electrometric investigations of small quantities of fluid, in cells and tissues.[54] The observation of the dielectric properties of fine particles in contact with various liquids has already been mentioned (page 146).

[49] Harrison: *loc. cit.*
Robinson: *loc. cit.*
Ambronn: *Koll. Zeits.* Spl. No. **36**, 119 (1925).

[50] Hepburn: *Jour. Chem. Soc.* **127**, 1007 (1925).

[51] Haber and Jaenicke: *Zeits. anorg. Chemie* **147**, 167 (1925).

[52] Brill and Evans: *Jour. Chem. Soc.* **93**, 1442 (1908).

[53] Mahin and Brewer: *Ind. Eng. Chem.* **12**, 1095 (1920).

[54] Ettisch and Peterfi: *Pflüger's Arch.* **208**, 454 (1925).
Taylor: *Proc. Soc. Exp. Biol. Med.* **23**, 147 (1925).
Taylor and Whitaker: *Protoplasma* **3**, 1–6 (1927).

Harvey[55] gives a complete discussion of various methods of determining the electrical conductivity of microscopic mineral grains, and shows how the information obtained can be used for identification purposes.

Electrolytic phenomena and the determination of hydrogen-ion concentration are discussed in Vol. II.

Magnetic properties of microscopic particles may be recognized by bringing a bar magnet near them. A magnetized needle or an electromagnet formed of a fine iron wire[56] will serve to pick out fine grains from mixtures. Grains in aggregates may be tested by bringing into contact with them a tiny horseshoe magnet made from a needle's eye suspended on a hair,[57] and noting whether it is attracted.

Observations of Photochemical Phenomena. — Alterations in the appearance of microscopic objects under the influence of light are significant and may usually be observed directly, by means of the illumination which effects the change. A sudden shift of the field will expose fresh material for comparison with that which has been photochemically influenced. The flocculation or precipitation of colloids by light, the "light-etching" of minerals,[58] the decomposition of silver halide grains in photographic emulsions,[59] and the reactions of plant and animal cells and organisms[60] are a few examples of possible applications and methods.

OBSERVATIONS OF THERMAL PHENOMENA

Thermal phenomena may be studied under the microscope with the aid of heating or cooling devices attached to the stage to permit regulation of the temperature of the specimen. Melting, freezing, transformation points (page 356), chemical reactions, and other phenomena affected by thermal conditions may thus be observed directly.

Hot stages consist of some form of compact heating device which will furnish the necessary temperature while permitting microscopic examination of the object. The temperature is held more or less constant, and some means of determining it is usually provided. Besides their application in the determination of fusion, subliming, and transition temperatures, hot stages are employed in the study of the gelatinization of starch, the vulcanization of rubber, coagulation of colloids, carbonization of coal or wood, decomposition of oil shales, chemical reaction at elevated temperatures, and the effect of heat on various sorts of materials.

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[55] *Econ. Geol.* **23**, 778 (1928).

[56] Taylor: *loc. cit.*

[57] McKinstry: *Econ. Geol.* **22**, 669 (1927).

[58] Whitehead: *Econ. Geol.* **12**, 907 (1917). McKinstry: *idem.* **22**, 669 (1927).

[59] Trivelli and Loveland: *Jour. Roy. Micros. Soc.* **272**, 293 (1925). Trivelli: *Jour. Phys. Chem.* **29**, 1568 (1925).

[60] Metzner: *Das Mikroskop* (Leipzig, 1928), pp. 346–50.

A great variety of types of hot stages have been devised, of which only those most suited to chemical work will be mentioned here.[61]

The simplest heating device consists of a tiny bent tube of glass, silica, or metal, temporarily attached to the substage ring and serving as a micro-burner.[62] The rotating stage is removed, and a piece of asbestos board, perforated at the center, is substituted. The specimen is placed on a thin slide, and the burner adjusted so that the flame is nearly in line with the axis of the microscope. By raising or lowering the substage the temperature may be regulated fairly accurately; it is estimated by observing the melting of standard substances (page 211). Chambers with air flues, heated by convection, have also been devised, to permit approximate temperature measurements by means of a thermometer.[63]

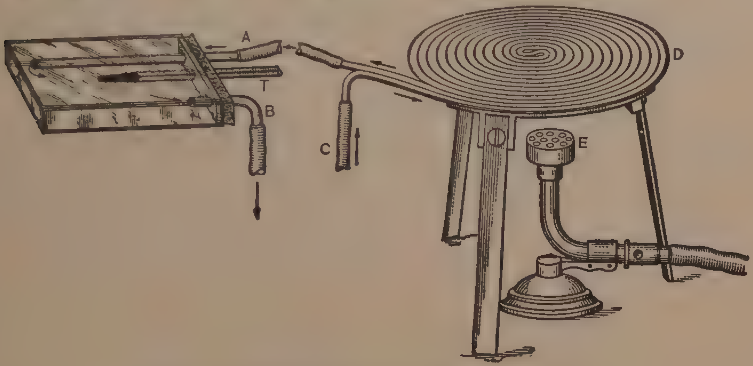

Fig. 87. Stage Heated by Circulating Liquid.

Temperatures below the boiling point of water may be attained by means of a hot stage through which hot water is made to circulate. A convenient form of apparatus[64] is shown in Fig. 87. It consists of a glass box or trough, such as is commonly employed for the spectroscopic examination of liquids, the open end of which is provided with a wedge-shaped piece of rubber, forming a tight stopper. Any flat-surfaced stoppered container may serve as a hot stage; for instance, a small flat bottle. The hot water enters the cell through the glass tube and escapes at *B*, the rate of flow being controlled by a stopcock or screw-clamp. The hot water may conveniently be obtained by siphoning it through a small coil of copper pipe *D* heated by a Bunsen burner *E*. Or the heating system devised for providing a continuous flow of hot water through a Zeiss butyro-refractometer may be employed. By regulating the heating flame and the rate of flow of hot water, very gradual or very rapid rises of temperature may be obtained or the temperature may be maintained almost constant. Jacketing the cell with asbestos simplifies the regulation of temperature.

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[61] See also Krause: *Enzyclopädie der mikroskopischen Technik*, Vol. I (1926), pp. 1268–77; Metzner: *Das Mikroskop* (1928), pp. 335–46.

[62] This heating device is essentially similar to that used for the study of crystallization phenomena by Lehmann: *Die Krystallanalyse* (Leipzig, 1891). See also Siedentopf: *Zeits. Elektrochem.* **12**, 593 (1906).

[63] Schwartz: *Gekrönte Preisschrift Göttingen* **1892.**
van Eyck: *Zeits. Phys. Chem.* **30**, 446 (1899).
Schaum and Unger: *Zeits. anorgan. Chem.* **132**, 91 (1923).
A steam-heated stage is described by Hauser: *Colloid Sym. Mon.*, **6**, 207 (1928).

[64] Chamot and Albrech: Unpublished paper presented to the Cornell Section, Amer. Chem. Soc., May, 1906. See also Francis and Smith: *Ind. Eng. Chem.* **8**, 509 (1916).

Heaters functioning on the principle of the thermo-siphon may also be employed for temperatures up to 85 to 90° C. Substituting brine or oil for water, the temperatures can be raised to 125–150° if the heating coil is used, but the viscosity of the oil is too great to permit an even and sufficiently rapid rate of flow unless large conducting pipes are employed, necessitating a cell far too thick for convenient use. The temperatures of the liquid may be conveniently measured by a thermometer, but since the specimen is commonly placed on the top of the cell its temperature will be somewhat lower than that indicated. If the character of the specimen permits, it may be immersed in the liquid, thus insuring much more accurate readings.[65]

Another readily improvised type of hot stage consists of a fairly thick plate of copper or aluminum, of such size as to fit in the frame of the microscope in place of the rotating stage, and pierced by an opening about 1 cm. in diameter. From this block projects a tongue of metal, which is heated by a gas flame.[66] By adjusting the burner, or varying its position, the temperature may be regulated (*cf.* page 138), and is reasonably constant on account of the good conducting properties and large heat capacity ("lag") of the piece of metal. Temperatures may be read by means of a thermometer inserted in a hole near the opening in the center of the stage. The material to be studied may be mounted on a slide which rests directly on the metal. For more accurate observation the sample may be placed between two cover glasses and held within the central opening in the metal plate by supports of bent wire; the top and bottom of the chamber may be closed by windows of glass or mica to render the interior temperature more uniform.

Electrically heated stages are more convenient and generally useful than other types. A wide range of temperatures may be obtained, control by means of a rheostat is easy, and the heat may be delivered to the object with little loss by radiation. The apparatus may be made simple and compact, to permit the use of objectives of relatively short working distance. In the design of an electrical hot stage, it is essential that its internal temperature shall be uniform, and this is best accomplished by distributing the heating element above as well as below the specimen. The temperature may be maintained more constant if the apparatus is heavily insulated, so as to possess considerable thermal "lag," but this may render it unduly slow in cooling.

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[65] An oil bath, containing a nichrome resistance for electric heating has been used in the thermal study of ammonium nitrate and other systems, the material being mounted on a slide and immersed in the oil. Bowen: *Jour. Phys. Chem.* **30**, 721 (1926).

[66] Schulze: *Arch. Mikr. Anat.* **1**, 1 (1865).
Gaubert: *Bull. Soc. fr. minéral.* **36**, 177.
Lenz: *Zeits. anal. Chem.* **52**, 90 (1913).
Stages of this type may be purchased from various makers.

Figure 88 shows an electrically heated hot stage that has been in use in the authors' laboratory for several years. It consists of a low cylinder of "Alberene stone" closed at the top and bottom by thin glass, or by mica when high temperatures are employed. The heating coil *H*, *H* consists of fine platinum wire wound in fine coils. In the illustration: *A* shows the Alberene stone; *B*, brass guides for the object slide acting as cover; *C*, adjustable wire fingers for supporting cover-glasses, tiny crucibles, "micro" retorts, etc.; *D* is a removable thin brass diaphragm cutting down the opening of the stage and serving as a radiator; *T*, thermometer; *PP*, binding posts; *M*, mica or glass window closing the bottom of the hot stage; and *S*, the object slide cover. The upper window of the stage consists of a thin glass object slip (or one of mica or of quartz) held in place by the guides *B*, *B*, permitting sliding the cover. This is essential when dealing with materials which sublime or give off steam, for in these cases the upper window becomes fogged with condensed material and in such an event the cover is simply pushed along until a clear section is obtained. The method of inserting the hot stage for use in place of the rotating stage is shown in Fig. 89. By attaching an Abbe drawing camera to the microscope tube and properly tipping the mirror, the image of the scale of the thermometer or galvanometer may be so reflected as to be seen alongside of the material whose melting point is to be determined. A lens attached to the body-tube or held in a separate stand serves to magnify the thermometer scale. It is thus possible to look into the tube of the in-

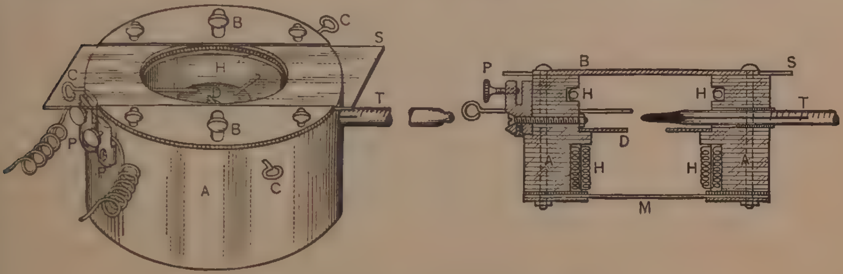

FIG. 88. Electrically Heated Stage.

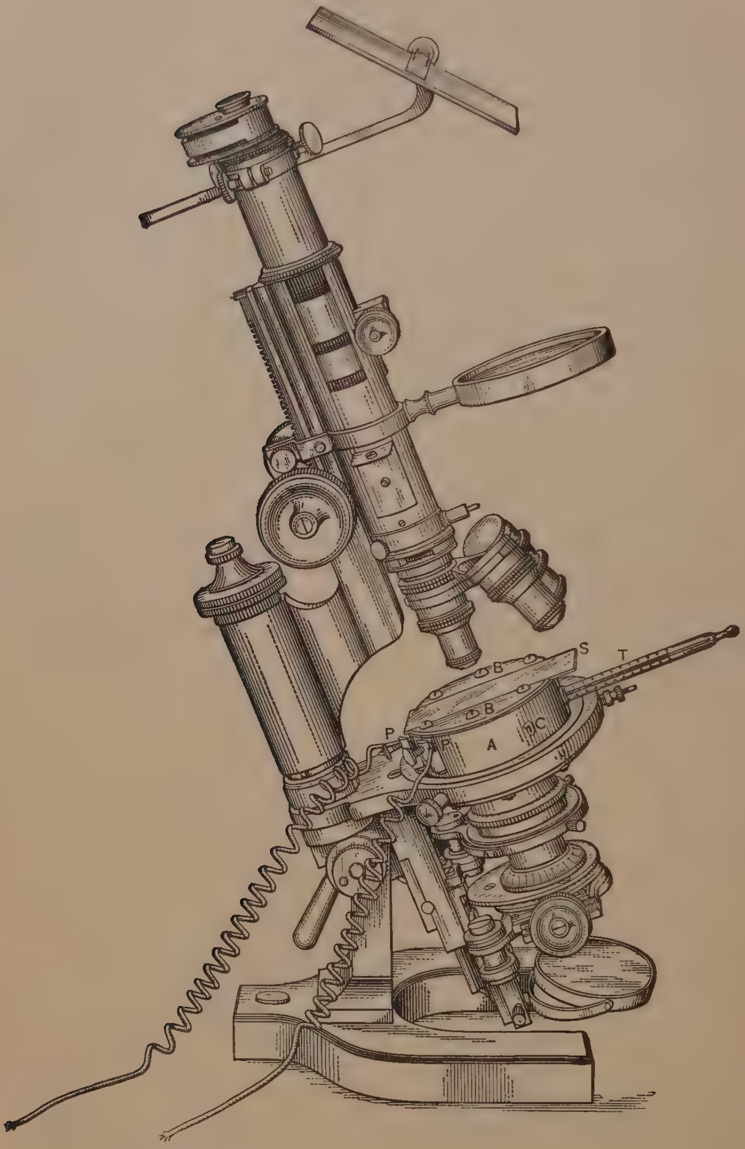

Fig. 89. Polarizing Microscope arranged for Observing Melting Points.

strument and to watch both the material and the thermometer. This arrangement and its applications will be readily understood by reference to the illustration.

The above stage is particularly convenient when fairly rapid heating or cooling is required; with platinum wire coils a temperature somewhat above 700° C. may be reached. A 16-mm. objective possesses a sufficiently long working distance to focus on the preparation through the cover-glass.[67]

An easily constructed hot stage is shown in Fig. 90. It consists of a block of aluminum, about 70 × 100 × 12 mm., perforated by an opening 19 mm. in diameter. The top and bottom surfaces are recessed, to receive thin glass windows, and a hole is drilled in one edge, to permit the insertion of a thermometer. About 2 meters of No. 20 (B and S) Nichrome wire is used as the heating element; it is wrapped around the aluminum plate in several layers, each of which is insulated by asbestos paper. In applying the insulation, space is left for the removal and replacement of the glass windows. Separate pieces of asbestos board, perforated with openings barely large enough to expose the specimen, are used as covers for these windows. The outer layers of insulation may be as thick as desired, so that temperatures may be held to ± 0.1° C. if the line current is reasonably constant. The interior of the chamber may advantageously be blackened, to increase the radiation by which the specimen is mainly heated. The material to be studied may be mounted between two 18-mm. round cover-glasses, and supported on a tiny wire tripod in the center of the heating chamber, practically in contact with the thermometer. Temperatures of 400° C. or more may be easily reached with this type of hot stage.[68] If an objective of very short working distance is to be used, the material may be mounted on a half slide which is put in place of the upper window. Temperature measurements are considerably less accurate under these conditions.

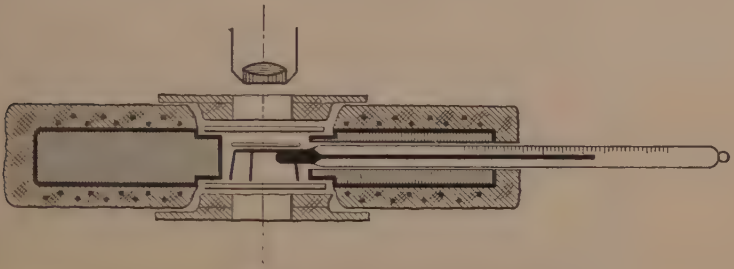

FIG. 90. Section of Electrically Heated Stage. × $\frac{2}{3}$.

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[67] A somewhat similar stage is described by Cram: *Jour. Amer. Chem. Soc.* **34**, 954 (1912). See also Walton: *Ind. Eng. Chem.*, (Analyt. Ed.) **1**, 106 (1929).

[68] An electrical hot stage of similar range is described by Clevenger: *Ind. Eng. Chem.* **16**, 854 (1924). See also Jentsch: *Zeits. wiss. Mikros.* **27**, 259 (1910); one of the simpler Jentsch hot stages is manufactured by Leitz.

Various types of electric "incubators" for use on the microscope are available for use at temperatures up to about 50° C.[69] They are provided with thermostatic control, and are particularly convenient for protracted heating.

Glass or quartz slides, on the surface of which a thin film of platinum[70] or silver[71] is deposited to serve as a resistance, have been used where a thin heating device is desired. Temperature measurements cannot be made directly, but only by comparison (page 208).

For very high temperatures, up to 1000° C. or more, special heating chambers are necessary, and care must be taken to protect the objective from radiated heat. Incandescent specimens are made normally visible by superposing an image of an arc lamp as a background, and reducing the brightness of the field by a dark glass screen above the eyepiece. Temperatures may be measured by an optical pyrometer[72] or a thermocouple. For observing fusion points of refractory materials, the sample may be placed directly on a narrow strip of platinum which is heated by a heavy current.[73] Chambers which are heated by resistance coils may also be used.[74] Some of these are inconveniently bulky for observations at moderate magnifications, but others permit the use of objectives of relatively short working distance ($<$ 16 mm. focal length).[75]

Cold stages consist of an insulated chamber which can be cooled below room temperature while permitting microscopic observation of the specimen. They are particularly useful in the study of melting and transition points and crystal properties of substances which are ordinarily liquid, and are also of value in observing the effect of freezing on emulsions, tissues, and other types of colloidal material.

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[69] Manufactured by the Chicago Surgical & Electrical Co., and obtainable from any of the larger dealers in laboratory apparatus.

[70] Cottrell: *Jour. Amer. Chem. Soc.* **34**, 1328 (1912).

[71] Metzner: *Jahrb. wiss. Bot.* **59**, 325 (1920); *Das Mikroskop* (1928), p. 339.

[72] Burgess: *U. S. Bureau of Standards, Bull.* **3**, 345 (1907); *Jour. Wash. Acad. Sci.* **43**, 7 (1913).

[73] Burgess: *U. S. Bureau of Standards, Circ.* **198**; *Jour. Franklin Inst.* **182**, 19 (1916).

Burgess and Wilttenberg: *U. S. Bureau of Standards, Bull.* **11**, 591 (1915).

Fieldner, Selvig and Parker: *Ind. Eng. Chem.* **14**, 695 (1922).

[74] Wright: *Jour. Wash. Acad. Sci.* **3**, 232 (1913).

Endell: *Zeits. Kristallog.* **56**, 191 (1921).

[75] Detailed descriptions of such heating stages are given by Oberhoffer: *Zeits. Elektrochem.* **15**, 634–46 (1909); Roberts and Stadnichenko: *Jour. Optical Soc. Amer.* **10**, 605 (1925); Eitel and Lange: *Zeits. anorgan. Chem.* **171**, 168–80 (1928).

The simple cold stage, shown in Fig. 91, may be made by any competent mechanism, and is suitable for cooling by various methods. It is constructed of a plate of aluminum about 70 × 100 × 10 mm.; the upper surface of this plate is channeled and a second plate, 3 mm. thick, is fastened tightly upon it by means of a leadfoil gasket and closely spaced screws. The channels thus constitute a tube for the circulation of a cooling agent through what is substantially a block of aluminum. An opening, 19 mm. in diameter, is provided for the specimen, and recessed at its edges to take windows of 25-mm. round cover-glasses, which are sealed in place by vaseline. A hole for the reception of a low-temperature thermometer extends into the central chamber. The whole stage is insulated by 5-mm. sheet cork, held in place by pins and rubber cement. Several layers of aluminum foil, cemented by rubber cement, protect the insulation from "sweating." The cold stage may be clamped on any microscope having a fairly roomy stand, preferably after removal of the rotating stage.

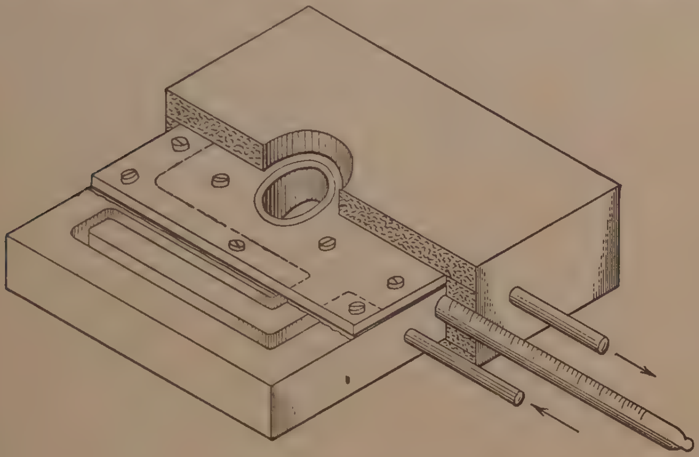

FIG. 91. Stage Cooled by Circulating Liquid.

The specimen is mounted in a small watch glass or between two 18-mm. cover-glasses, and is supported by a tiny wire tripod in close proximity to the bulb of the thermometer. An objective of 16-mm. focal length may be used, and by placing the specimen near to the upper window even higher powers may be focused.

Cooling is effected by gravity circulation of alcohol, which passes through a spiral of copper tubing and thence by a rubber tube to the stage. The copper spiral is immersed in a bath of "carbon dioxide snow" in alcohol or ether, contained in a Dewar beaker. By regulating the rate of flow, the temperature of the stage may be adjusted, and held constant within 1° C. or less. Temperatures as low as $-40°$ C. can be reached by this arrangement, and by using liquid air as a cooling bath, still lower temperatures are possible. The stage may also be cooled by the vaporization of liquid carbon dioxide, a cylinder of which is connected to it by copper tubing. The flow of the liquid is regulated by a valve just outside the stage, so that the expansion takes place within the channel. By the circulation of warm water, the stage may be used for heating purposes, up to about 100° C.

At moderately low temperatures, in a dry atmosphere, condensation on the outer surface of the glass windows is not serious, but if the air is humid droplets of moisture collect and interfere with the visibility of the specimen. A film of glycerin on the glass will obviate this difficulty. A heat-absorbing glass filter (page 108) is essential if the specimen is to be illuminated by a powerful light source.

The above stage is satisfactory for ordinary investigations, and is much more compact than others which have been devised primarily for very low temperatures.[76]

Measurement of temperatures in hot or cold stages may be carried out by a number of different methods. For accurate work it is essential that the observed temperature shall correspond to that prevailing in the immediate vicinity of the specimen. A well insulated chamber, close proximity of specimen and measuring device, and slow heating or cooling will all help to insure this.

Substances of known melting point may be used as standards and as checks on the accuracy of thermometric measurements in hot stages.[77] Tiny fragments of one or more reference substances may be placed on the slide adjacent to the specimen which is under observation, so as to be heated exactly similarly. This method is particularly valuable in comparing melting points of known and unknown materials, especially when a poorly insulated hot stage is used.

Thermometers are most convenient for measuring temperatures in hot or cold stages, and are satisfactorily accurate if the proper precautions are taken. Anschütz thermometers are preferable for hot stages, as stem correction is not necessary and their graduations permit readings to 0.1°. The bulb of the thermometer should be well inside the chamber, and as close to the specimen as possible. Heavy insulation of the stage is desirable, and heating or cooling should be slow enough to insure uniform temperature.

A thermocouple has the advantage that its hot junction is smaller than a thermometer bulb, and may be placed in close contact with the specimen. The "lag" is likely to be less than that of a thermometer and a greater range of temperatures may be measured. Iron or copper, with "constantan," or "Chromel X" with "Copel," are most suitable as materials for thermocouple to be used at moderate temperatures. The cold junction of the couple can be kept at constant temperature by immersing in a Dewar beaker filled with melting ice. A sensitive millivoltmeter or a potentiometer is used for measuring the potential produced.

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[76] Backe: *Jour. Roy. Micros. Soc.* **1909**, 768; *Zeits. Instrumentenkunde* **29**, 72 (1909); *Zeits. wiss. Mikros.* **26**, 166, 587.

Vorländer, Selke, and Kreiz: *Berichte* **58**, II, 1802 (1925).

The optical study of solidified gases has been carried out by Wahl: *Proc. Roy. Soc.* (London) **874**, 371 (1912), who observed their crystallization in a thin quartz cell immersed in liquid air, by means of a horizontal microscope.

[77] A table of melting points of pure substances suitable for these purposes is given on page 211.

The thermocouple must be calibrated by means of compounds of known melting points, such as are given in the Table on page 211. The melting points of these standards are first determined by the usual method, in small melting point tubes. Then each of them in turn is heated under the microscope, and a reading of millivolts is taken as it fuses. A curve of millivolts against temperatures is thus prepared, to be used for future determinations under similar conditions.

Determinations of melting points under the microscope may be carried out on minute amounts of material, with a high degree of accuracy. The phenomena of fusion are directly observable, and the transition from the solid to the liquid state is unmistakable. Except in the rather uncommon cases of isotropic solids or "liquid crystals," the *disappearance of double refraction* constitutes a very positive and exact criterion of melting, which is readily utilized by means of the polarizing microscope[78] (Fig. 89). Strictly speaking, fusion of crystalline material involves the destruction of its space lattice to which its double refraction is inherently related.

Non-crystalline substances do not possess a true melting point, but their softening to a fluid condition may be observed very readily by means of the microscope. Mixtures of crystalline and non-crystalline materials, such as oils, fats and waxes, exhibit incipient fusion which is easily determined. The range of temperatures between initial and complete melting of mixtures is easily determined. Most of these phenomena are masked by ordinary methods of melting point determination. Because of the ease with which melting is rendered visible, microscopic methods generally give values which are slightly lower than those obtained in the usual way. This should be borne in mind in calibrating thermocouples and in making comparisons with published data.

The material to be tested, preferably well crystallized, may be supported on a thin cover glass or contained in a tiny glass capillary tube. For fats and waxes the tube may be inclined and the melting point taken the instant the sample slides out of focus. With any type of hot stage it is advisable that a preliminary observation of the melting point should be made, to be followed by a second determination under carefully controlled conditions. The temperature should be raised very gradually as the melting point first observed is approached, and should be held constant as soon as fusion begins, so that solid and melt co-exist in equilibrium. By raising or lowering the temperature a fraction of a degree, either phase may be made to grow, and the melting point may be approached from either side without risk of supercooling. Under these circumstances the effect of lag in the thermometer or thermocouple is at a minimum, and with a well insulated hot stage readings should be accurate to about 0.1° C.

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[78] The nicol prisms should both be rotatable, preferably by means of a connecting bar.

In determining freezing points by means of a cold stage, particular care must be taken to avoid supercooling, and the temperatures of solidification and of melting should both be ascertained.

TABLE I

MELTING POINTS OF COMPOUNDS USEFUL FOR APPROXIMATE MELTING POINT DETERMINATIONS WITH THE MICROSCOPE

Melting Point C°.*	*Compound*
20	Acetophenone
22	Anethol
27	Diphenylmethane
30	Orthocresol
35	Paracresol
41	Phenol
43	Salol
45	Orthonitrophenol
48	Chloralhydrate; Urethane
50	Alphanaphthylamine
52	Thymol
53	Paradichlorobenzene
58	Trichloracetic acid
63	Metaphenylenediamine
67	Coumarin; Azobenzene
69	Diphenyl
70	Pyrazole
72	Orthonitroaniline
74	Hedonal
75	Borax
76	Trional
80	Naphthalene
81	Vanillin
86	Saligenin
87	Paradibromobenzene
90	Metadinitrobenzene
93	Triphenylmethane; Salipyrin
96	Alphanaphthol; Metanitrophenol
100	Phenanthrene; Exalgin
104	Orthophenylenediamine
105	Pyrocatechin
108	Pyramidon
110	Resorcinol; Betanaphthylamine
112	Metanitroaniline
113	Paranitrophenol
114	Acetanilide; Ammonium acetate
116	Atropine; Quinone
117	Orthodinitrobenzene
118	Chrysoidine
119	Iodoform
122	Benzoic acid; Picric acid; Betanaphthol
123	Dionine
128	Sulphonal
129	Orthotolidine
131	Maleic Acid
133	Urea; Hydrastine
134	Pyrogallol
137	Picrolonic acid
140	Paraphenylenediamine
145	Anthranilic acid
148	Paranitroaniline; 2, 4-dinitroresorcinol
150	Ammonium thiocyanate
153	Citric acid; Methylglyoxime
156	Benzenesulphonimide
159	Salicylic acid
164	Cupferron
169	Hydrochinon
170	Santonin
171	Dimethylamine hydrochloride
172	Paradinitrobenzene
173	Potassium thiocyanate
175	Quinine; Narcotine
178	Brucine
184	Quinine citrate
185	Succinic acid; Cinchonamine
186	Saccharose
189	Nitron
191	Veronal
193	Chrysophanic acid
198	Aniline hydrochloride
202	Salicin; Lactose
210	Chinconidine
212	Silver nitrate
218	Anthracene
219	Phloroglucinol
228	Saccharin
235	Carbanilid
237	Caffeine
244	Carbazole
246	Dimethylglyoxime
248	Sodium chlorate
261	Phenolphthalein
268	Strychnine
280	Lead acetate (anhydr.)
285	Anthraquinone
288	Paraaniline sulphonic acid
290	Alizarin
297	Potassium nitrite
302	Mercurous chloride
324	Sodium acetate (anhydr.)
337	Theobromine
368	Potassium chlorate
398	Potassium bichromate

* The melting points given in this table have been compiled from *International Critical Tables*, National Research Council (McGraw-Hill Book Co., New York, 1926). Figures are given to the nearest whole number. Melting points as observed under the microscope are apt to be several degrees lower than those recorded above.

CHAPTER VII

ULTRAMICROSCOPY

It has already been pointed out (page 94) that dark field illumination, if the radiant is sufficiently intense, will reveal the presence of particles which are far beyond the resolving power of the microscope. The increase in visibility is due to the fact that the light scattered by the particles, faint though it may be, is seen against a black background and is more readily perceived by the eye. This is analogous to the scintillations from floating dust particles, ordinarily invisible, which are so easily seen in a sunbeam entering a darkened room. The ultramicroscope is an adaptation of this phenomenon, by means of which a specimen containing suspended particles may be examined by an ordinary microscope while illuminated as indicated above.

If a powerful beam of light is directed through a suspension, its path will be visible by virtue of the light scattered by the particles which it encounters. This phenomenon is known as the " Tyndall effect," and is employed directly in the slit ultramicroscope, and in a modified form in the various types of ultramicroscopic condensers.

Although the existence of particles in colloidal solutions had been inferred by Faraday, it was not until much later that Zsigmondy and Siedentopf actually demonstrated the reality of such particles under the ultramicroscope. Some form of ultramicroscope is almost indispensable in any laboratory where work is being done on colloidal materials, for in almost all cases it gives immediate positive or negative evidence of the colloidal nature of a given preparation.

When particles or other fine structures are not too small, their individual surfaces will be resolvable by the microscope, and they will appear to have a definite form, self-luminous against a dark ground. If their dimensions are so minute that their bounding surfaces are not seen as separate, they will appear as tiny spots of light. As mentioned above (page 13) these are not points, but diffraction disks surrounded by one or more alternate light and dark rings. The size and shape of the diffraction disk is practically independent of the size and shape of the particle, and it does not constitute an actual image. However, the appearance of such diffraction disks in a properly illuminated specimen is evidence of the presence of minute particles, sometimes called submicrons, ultramicrons, or micellae.

The microscope by which these diffraction patterns are viewed serves only to reveal their number and distribution; it is usually of moderately high power. The particles must not be too close together in the specimen or their diffraction patterns will be merged and they will not be resolved as individuals. Except for this qualification, the resolving power of the ultramicroscope is practically unimportant, and its remarkable capabilities are mainly dependent upon the illuminating system.

The efficiency of any ultramicroscope as a means of revealing submicroscopic particles is governed by the following factors:[1]

1–The intensity of illumination.
2–The aperture of the illuminating and of the imaging lenses.
3–The darkness of the field.
4–The diffracting power of the particles.

The dependence of each of these on manipulative technique and instrument design will be discussed in the pages which follow.

UNILATERAL ILLUMINATION

The slit ultramicroscope of Siedentopf and Zsigmondy[2] may be said to be the earliest practical instrument for the study of ultramicroscopic particles. It supplies **" orthogonal " illumination** in a narrow intense beam perpendicular to the axis of the microscope, and at first sight might be thought to be the most efficient type, since the more nearly the illumination is perpendicular to the axis of the microscope the stronger the diffraction phenomena. However, light comes to the submicroscopic particles from one direction only, and of necessity cannot illuminate them as powerfully as if it converged upon them from all sides. The slit ultramicroscope is particularly suited to studies of the size, shape, and other properties of colloidal particles whether in solids, liquids, or gases, on account of the excellent control of the illuminating beams, made possible by an elaborate collimating system.

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[1] Siedentopf: *Zeits. wiss. Mikros.* **26**, 391 (1909).

[2] *Annalen der Physik* (4) **10**, 1 (1903); *Jour. Roy. Micros. Soc.*, **1903**, 5–73.

The form and arrangement of the component parts of slit ultramicroscopes differ according to the maker.[3] The best known type is shown in Fig. 92. It consists of an optical bench *B*, at one end of which is placed an arc lamp *R* (an automatic arc is commonly supplied) and at the other a compound microscope *M*. The light from the crater of the arc is collected by a corrected lens C_1, of 120-mm. focal length and imaged on the slit *S*. The opening of this slit is ordinarily adjusted so as to extend horizontally, its ends being limited by the screw *s* and its width by the micrometer *G*. The slit is essentially a field diaphragm, since it serves to regulate the cross-section of the illuminating beam. The lens C_2 (55-mm. focal length) projects an image *F* of this illuminated slit at a distance of 160 mm. from the objective C_3, and may be moved on its support to adjust the size of this image. The objective C_3 (16-mm. focal length) reduces this image of the slit and projects it in the plane of the optic axis of the microscope *M*. The depth and width of the illuminated layer of material in the cell *U* are regulated by the screws *S*, *s* which control the size of the slit.

The objective C_3 may be moved along its axis in a sliding sleeve *T*, and by a fine adjustment screw V_1. Lateral motion is controlled by the screw V_2. An adapter *A* with centering screws *a* may be used to align the objective *O*. The microscope is equipped with a mechanical stage *P* which is movable forward and back by the screw W_2, laterally by the screw W_1, and may be raised or lowered by the nut *q*. The object under examination (as shown, liquid in a Biltz cell), is held by the spring clip *p*, and may be accurately moved in any direction either for centration or to present new portions to the illuminating beam.

Adjustment of the slit ultramicroscope is easily carried out if the functions of its various parts are kept in mind. A specimen which shows pronounced light scattering properties[4] should be kept at hand as an aid in aligning the illumination. The positions of the various parts of the illuminating train are marked on the optical bench, and if they are so placed and the arc lighted, its image may be located by holding a piece of black paper or ground glass near the slit *S*. By sliding the lens *C* forward or back, this image may be definitely focused. If it is not centered so that the crater of the arc covers the slit, the position of the arc should be adjusted. The automatic arc which is commonly supplied with the instrument has centering screws for this purpose.

The microscope is placed on its base, but not clamped tightly at first. The specimen is placed beneath the objective *O*, approximately in focus. If the objective C_3 and the collimating lens C_2 are in place, a beam of light (which may be located by a piece of black paper) will be sent horizontally across the stage of the microscope. All the adjusting screws should be turned to the middle of their range, and the microscope should be moved on its base plate until the beam is approximately centered, and is visible in the specimen, either with the naked eye or with the micro-

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[3] There are but three models of slit ultramicroscopes on the market at present that are suitable for general chemical investigations; these are made by Zeiss, Leitz, and R. & J. Beck. The Bausch & Lomb Optical Co. have in process of development a slit ultramicroscope that gives promise of excellent results.

[4] Such as the gold ruby glass supplied by Zeiss.

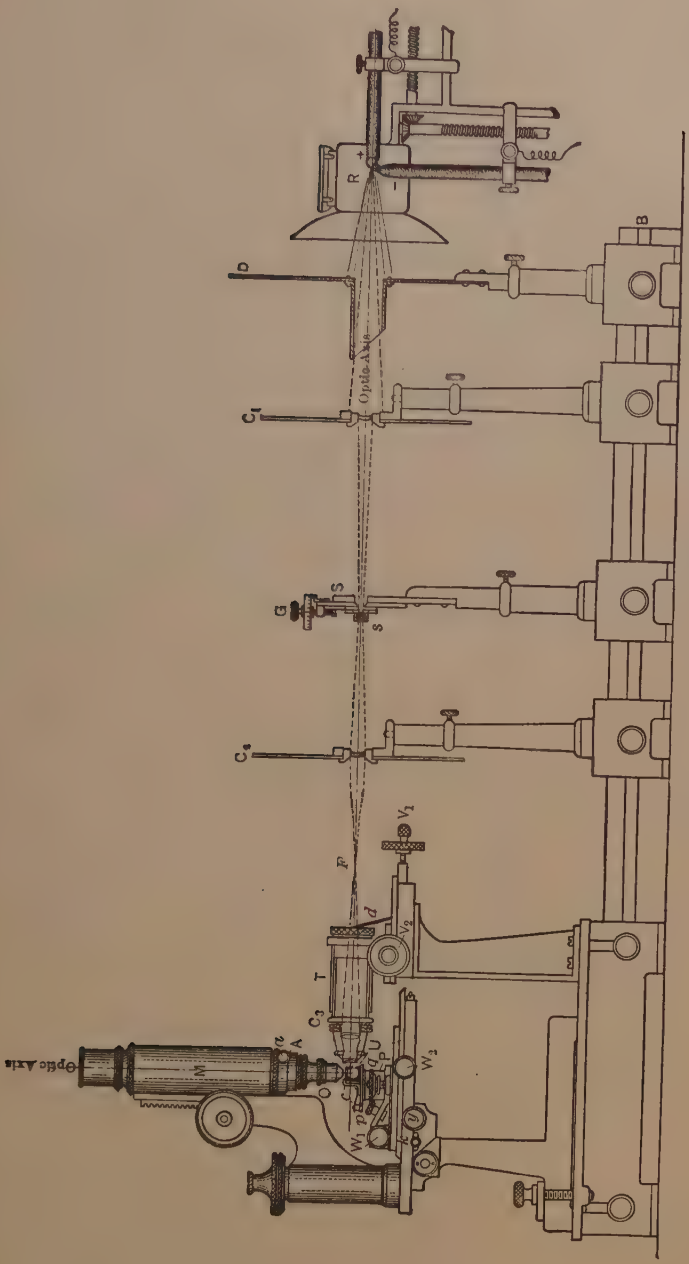
Optic Axis
M
a
A
O
C
U
q
p
P
W1
W2
y
t
C3
T
d
V2
F
V1
C4
G
S
s
C1
Optic Axis
D
R
+
−
B

FIG. 92. Slit Ultramicroscope. (Zeiss.)

scope. The microscope may then be locked in place, and the beam adjusted laterally by means of the screw V_2, so that it passes across the middle of the field of the microscope. By moving the elevating nut *q*, the specimen may be brought to the proper level, so that the beam enters its vertical face. Now the microscope may be focused, and the narrowest portion of the beam brought to the center of the field by moving the objective C_3 along its axis by the screw V_1. Ideally, the specimen should be so placed that the light converges just below its surface, and just inside its vertical boundary. Slight changes in the focus of the microscope and of the objective C_3 may be necessary when this adjustment is made by means of the mechanical stage, on account of the alteration of the distance the light travels in the specimen.

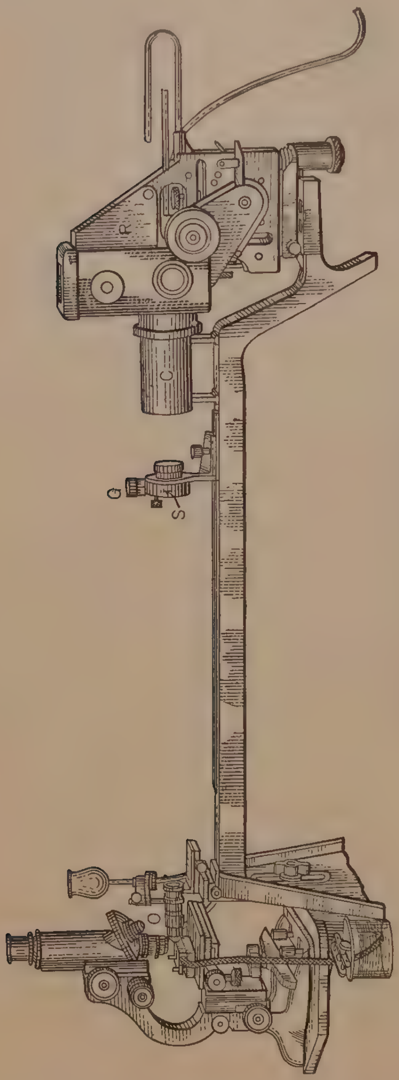

FIG. 93. Slit Ultramicroscope. (Leitz.)

The slit may be adjusted to give a beam of the desired width by the screw *s*; it may then be rotated 90° about the axis of the beam and the thickness or depth of the beam adjusted by the micrometer screw *S*. When turned back to its original orientation the slit furnishes a shallow beam, which may be made only a few microns in thickness. Its exact dimensions may be measured by means of a calibrated scale in the eyepiece. When dealing with exceedingly fine colloidal particles it is sometimes desirable to cut off the lower half of the beam by laying against the end of the tube *T* a small rectangular piece of black opaque material *d*.

A new and somewhat simplified slit ultramicroscope has recently been placed on the market by Leitz (Fig. 93). It is essentially similar in principle to the instrument just described, but of simpler construction in that the reduced image of the slit *S* is formed by the objective *O*, no other lenses being employed. The opening of the slit is adjustable, and the cross-section of the illuminating beam may be reduced to a minimum by sliding it as far away from the objective *O* as the optical bench permits; the objective is provided with a correction collar to compensate for the change in distance. The condenser *C* of the automatic arc lamp *R* is not adjustable, and the crater can be imaged on the slit only when the latter is brought close to the microscope. Coördinate movement of the specimen is effected by means of a microscope stand with a focusing stage *S* having two horizontal movements. If an ordinary stand is used, an auxiliary elevating stage may be attached to a Leitz mechanical stage, to give movement in three directions.

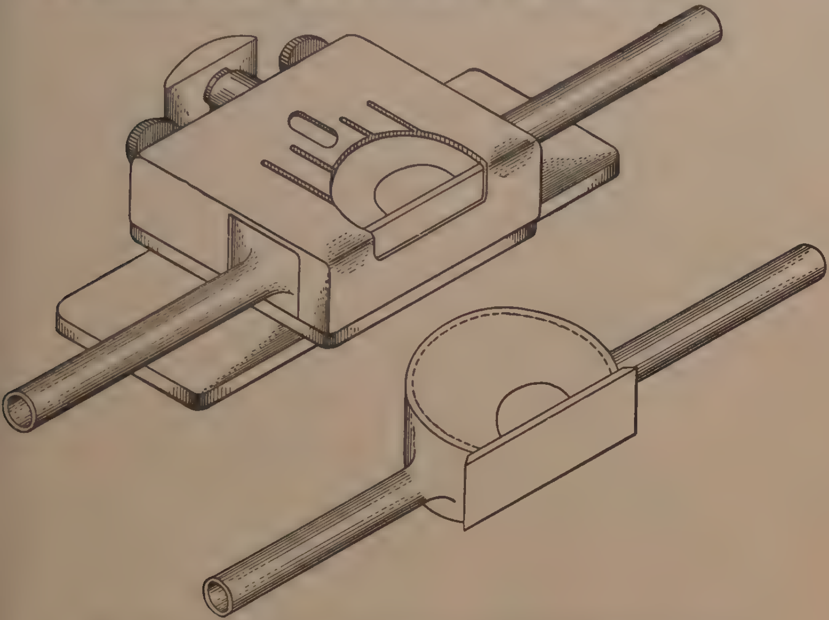

Fig. 94. Quartz Cell and Holder, for Leitz Slit Ultramicroscope.

The quartz cells used with this instrument for the study of liquids are of the type shown in Fig. 94. They are held against the front window by a spring clamp, the cover-glass being kept in place by a housing fastened by two set-screws. No cement is used, capillarity being supposed to hold the liquid in the cell, but actually leakage is likely to occur. The cell shown in Fig. 97 may be used with this ultramicroscope and is preferable to the type supplied with it, on account of its ease of filling and cleaning and simplicity of construction.

A very simple though moderately effective ultramicroscope for orthogonal illumination, which may be readily improvised from almost any ordinary microscope, is described by Kiplinger.[5]

[5] *Jour. Amer. Chem. Soc.* **39**, 1616 (1917). See also Doane and Dow: *Jour. Chem. Educ.* **6**, 1099 (1929).

The "Brownian Movement Apparatus" sold by the Central Scientific

Cells for holding fluids to be studied with the slit ultramicroscope are made so as to present a plane vertical surface for the entry of the illuminating beam, and a plane horizontal surface for the exit of diffracted light to the microscope.

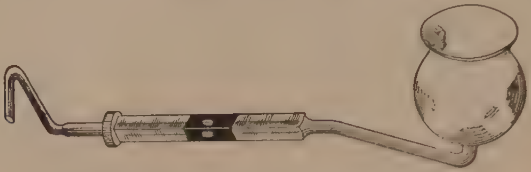

Fig. 95. Biltz-Thomae Cell.

The Biltz-Thomae cell (Fig. 95) consists of a filling tube and trap, between which is a cell with a central portion of black glass. In this are two small windows (Fig. 96) of thin glass, or of quartz. The illuminating beam *IS* is in part diffracted by colloidal particles; the remainder is absorbed by the black glass walls of the cell *P*. The microscope, in the optic axis *OA*, will therefore receive only light from the particles.

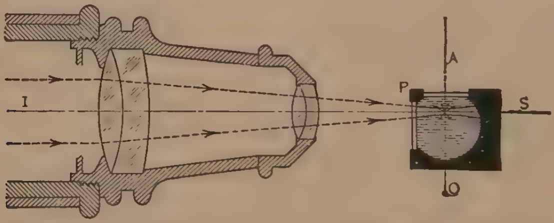

Fig. 96. Illuminating Rays in the Cell of the Slit Ultramicroscope.

The special holder for Biltz cells, as furnished with the ultramicroscope, is distinctly inconvenient to use, since it is attached to the objective. Centration, focusing, or interchange of cells are very difficult, especially since the cells are seldom of exactly uniform dimensions. It is much preferable to support the cell upon the elevating mechanical stage *P*, as shown in Fig. 92 by means of modified spring clips. This arrangement permits movements in all directions, and centration of the windows with the objectives *O* and C_3 is easily accomplished. One of the most serious defects of the Biltz cell is the difficulty of cleaning it after use, especially when there has been a

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Co., though hardly entitled to be called an ultramicroscope, deserves mention here as an effective device for demonstrating Brownian movement in smokes. A microscope magnifying at least 100×, a light source with condenser giving a concentrated beam (such as the lamp in Fig. 43) and the apparatus itself, costing $4.50, constitute the necessary outfit.

deposition of a colloidal film upon the windows. Bubbles are easily entrapped in filling, and collect at the window openings.

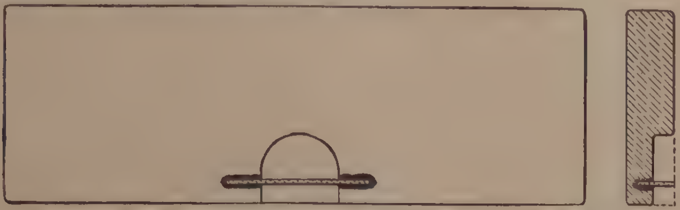

Fig. 97. Simple Cell for Slit Ultramicroscope. (Full size.)

A much cheaper and simpler cell, suitable for study of all sorts of corrosive liquids such as are encountered in chemical work, is shown in Fig. 97.[6] It consists of a piece of glass similar to a microscope slide, about 5 mm. in thickness. In one edge of this a recess is ground, and a smooth vertical window cemented in place in a groove parallel to the front edge of the slide. An ordinary square cover-glass is used to enclose the top of the cell, which is easily filled with a pipette and may be thoroughly cleaned by washing and wiping if necessary. A few tiny bubbles do no harm if they are well outside the path of the illumination. A number of these cells should be kept on hand, so that comparative studies on several samples may be readily made.

Fragmental or irregular specimens of solid colloids may be immersed in a cell containing a liquid of their own refractive index, and examined similarly. Where a sufficiently large piece of solid material is available, it is better to shape it so that two plane adjacent mutually perpendicular faces are available (Fig. 98). If these faces do not meet in an edge, as at *a*, the illuminating beam *R* must be lowered to clear the irregular portion *b*, and this may place it outside the working distance *W* of the objective *O*. The labor of grinding and polishing specimens to the form indicated may sometimes be eliminated if smooth fractured faces, meeting at approximately 90°, can be found. Ce-

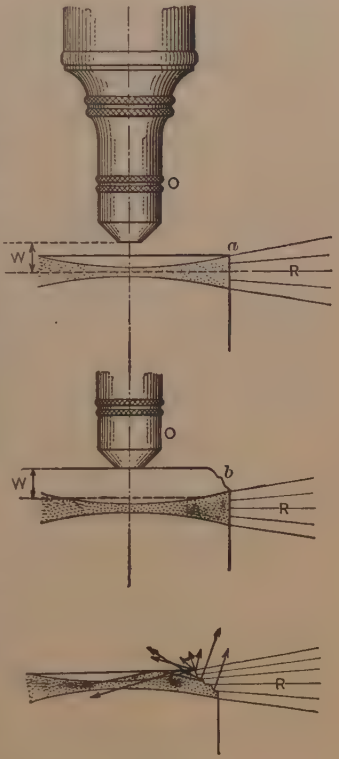

Fig. 98. The Necessity of having Two Sides at Right Angles in the Object for Ultramicroscopic Study.

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[6] Made by Zeiss.

menting a small piece of cover-glass on the top and side of a rough specimen may eliminate the scattering of light by surface irregularities sufficiently for ordinary examinations.

The Immersion Ultramicroscope. — The orthogonal beam of the slit ultramicroscope is very narrow, the numerical aperture of the illuminating objective being about 0.3. In order to supply a higher intensity of light to the part of the specimen which is being studied, Zsigmondy[7] devised an instrument in which an objective of much higher numerical aperture is used to converge light in an obtuse cone upon a small region of the object under examination.

In this new Immersion Ultramicroscope[8] both the illuminating and observing objectives are beveled at the ends so as to allow their front lenses to be brought very close together with their axes at right angles; the drop of liquid to be examined is placed between the front lenses, clinging by capillarity. No cell is employed. The light rays having but a very short distance to travel, even dark colored liquids may be studied. Difficultly cleanable, expensive cells are thus wholly eliminated, the amount of material required for study is reduced to a minimum, and the images obtained are exceptionally brilliant.

For the study of hydrosols, water immersion objectives must be used, but for colored glass and similar bodies homogeneous immersion objectives are required.

The construction of the instrument is shown in the diagram, Fig. 99. Fitted to the body-tube of a compound microscope is the objective carrier C into which slides a plate to which is screwed the image-forming objective O. To the stage of the instrument is attached the mechanism supporting the illuminating objective I. The micrometer screws S^1, S^2, S^3 provide means for the exact adjustment of the beam of light passing in the line of the axis of the objective I, so that it will fall normal to the optic axis of the microscope. S^1 gives an up-and-down adjustment, S^2 forward-and-back and S^3 from side to side. By rack-and-pinion S^4, the entire illuminating device can be lowered for cleaning, for the removal of the objectives, etc. When raised in position for use, the screw s is turned, thus locking the mechanism in place.

The trough T serves to catch any drip when the liquid is being applied between the objectives. A cell is provided to cover the objectives and liquid and prevent convection currents.

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[7] *Physik. Zeits.* **14**, 975 (1913).
Zsigmondy and Bachmann: *Koll. Zeits.* **14**, 283 (1914).
King: *Jour. Soc. Chem. Ind.* **38**, 4 (1919).
Zsigmondy: Alexander's *Colloid Chemistry* (1926), p. 817.

[8] Made by C. Winkel, Göttingen, Germany. Now associated with Zeiss.

When in use, the instrument is placed on a bed plate with saddle stand, upon an optical bench. An apparatus consisting of a condensing lens and an adjustable slit, also on saddle stands, serves to throw a beam of light from a radiant into the objective *I*.

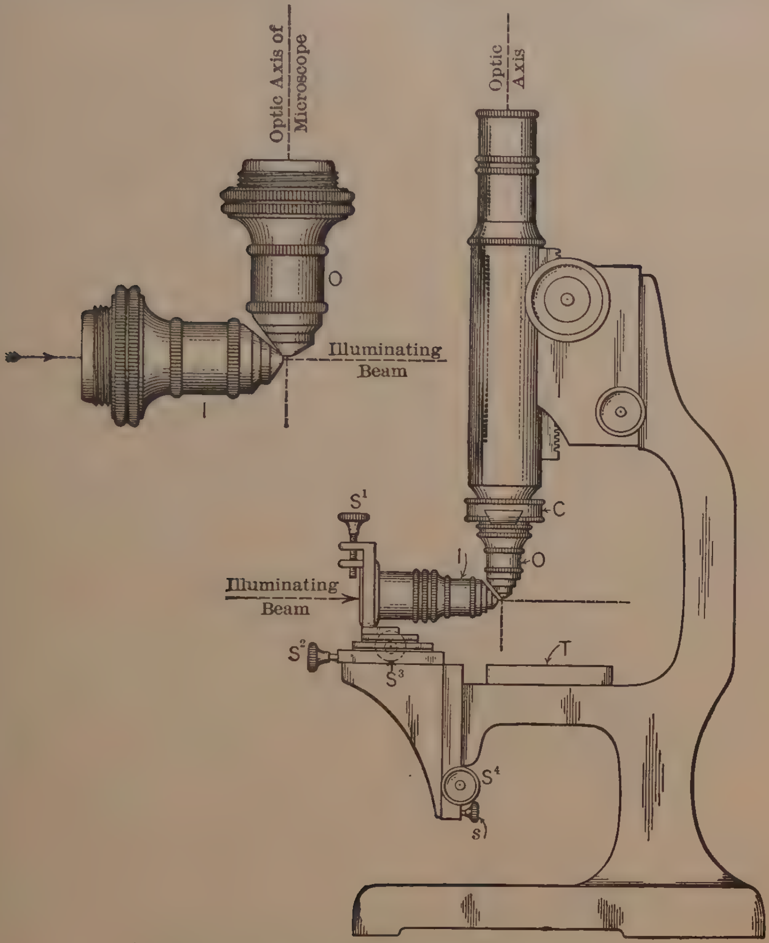

FIG. 99. Zsigmondy Immersion Ultramicroscope.

In critical work the ocular of the microscope is furnished with an adjustable slit-diaphragm, thus permitting the cutting down of the field until only a certain selected portion is visible.

The mutual arrangement of the two objectives is shown in the diagram.

These objectives embody several unique ideas in mounting, construction and in the component lenses themselves; the end, or front, lenses are of quartz. An examination of the diagram will show that a drop of liquid brought into contact with the two front lenses will cling in place. The illuminating beam will pass through this drop in the focal plane of the objective *O*. The image resulting upon focusing the microscope will appear to be two hazy triangles of light united at their apices by a more or less marked brighter thread or band. In this band are seen the diffraction disks due to the infinitely small particles in suspension. By means of an ocular diaphragm all of the hazy triangles are cut off and the connecting thread or band of light alone allowed to appear in the field of view.

By the use of the immersion ultramicroscope it has been possible to observe particles which practically mark the limit of ultramicroscopic visibility. However, for general work the slit ultramicroscope is preferable, since it can be used for solids or for corrosive solutions.

ANNULAR ILLUMINATION

In ultramicroscopy with annular illumination the light converges upon a small area of the specimen in the form of an obtuse hollow cone, as has already been discussed in connection with dark field illuminators (page 86). Because of its incidence from all sides, a high intensity of illumination is secured, and exceedingly fine particles are revealed.

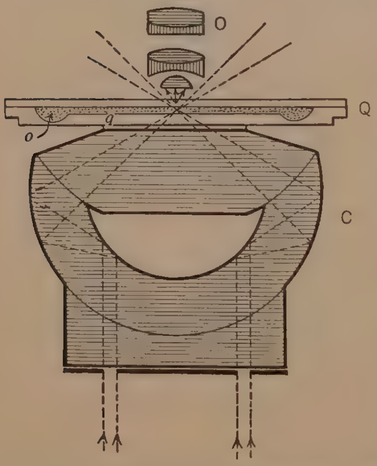

FIG. 100. "Cardioid" Condenser and Cell for Specimen.

The "Cardioid" ultramicroscope of Siedentopf[9] (Fig. 100) consists of a condenser in which reflection takes place at two spherical[10] surfaces and highly perfect union of the rays is accomplished. This condenser *C* is mounted in the substage ring[11] of an ordinary microscope *M*, and is illumi-

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[9] *Verh. der Deutsch. physik. Ges.* **12**, 6 (1910). Manufactured by Zeiss, and also by Bausch & Lomb.

[10] Ideally, one of these should be a cardioid of revolution, but practically this is not necessary, and would be very difficult to construct.

[11] The Zeiss cardioid condenser is not of standard diameter, and requires an adapter if it is to be used on other than Zeiss stands.

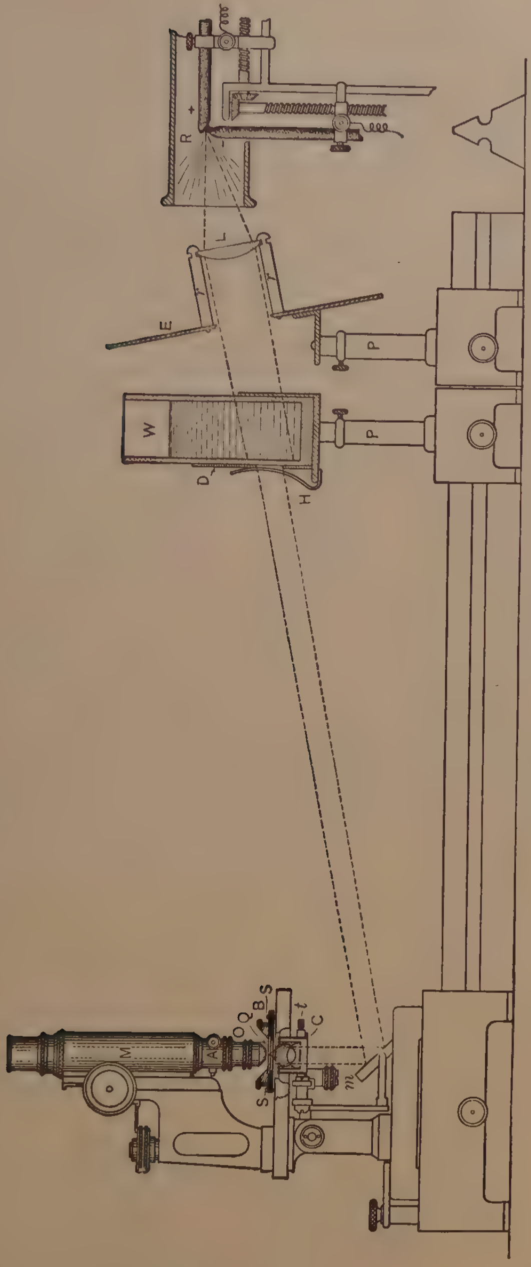
R
L
E
W
D
H
P
P
M
A
O
Q
B
S
S
t
C
m

FIG. 101. Cardioid Ultramicroscope.

nated by a powerful arc R, as shown in Fig. 101. An optical bench is desirable but not essential. The liquid to be examined is contained as a thin film in a special quartz cell Q, held in a brass mounting B, on the stage of the microscope.

Parallel rays from the arc, or else an image of its crater, are delivered to the back aperture of the condenser by means of an auxiliary condenser and the plane mirror of the microscope. The top of the condenser is made level with the surface of the stage, and brought into optical contact with the lower surface of the cell by a thin film of glycerine or of water.

The liquid to be examined is placed in the quartz cell Q (Figs. 100, 102) which consists of a disk with a central plateau q surrounded by a groove o. This plateau is about 2 microns lower than the outer edges of the disk, so that a shallow space is left between it and the cover when the latter is pressed down by the ring f and the screw ring b. By this means a thin and uniform layer of liquid is obtained, the surplus being forced into the groove o. The thickness of the film of liquid can be measured by means of a graduated fine adjustment on the microscope, taking into account its refractive index (see page 408). Special perforated covers can be obtained for the introduction of reagents during examinations.

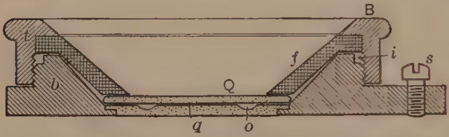

Fig. 102. Holder and Cell for Liquids, for use with the Cardioid Ultramicroscope.

It is absolutely essential that both cell and cover be absolutely clean and free from dust or films of grease. Hot cleaning mixture, followed by rinsing in distilled water and then in really clean alcohol, will render the surfaces fairly clean, but any wiping or exposure to the air will permit them to gather dust. They should be dried in a current of warm air and burned clean by heating to a red heat in the flame of a Bunsen burner. As soon as they are cool, they should be placed in the holder and used at once. An alternative method of cleaning is to coat the surfaces with collodion, which is allowed to dry, and is stripped off just before the cell is used, taking all dust particles with it.

The cell holder (Fig. 102) has an unnecessary number of threads, and all but three turns may well be removed, as shown at i, to save time in emptying and filling the apparatus. Leveling screws s are provided to adjust the height of the cell above the stage, and to render it perpendicular to the axis of the microscope. A centering adapter A, or else a centering substage, is essential in order that the field of the objective O shall be exactly coincident with the small area illuminated by the condenser.

A special 3-mm., 0.85-N.A. glycerine immersion objective is supplied, to give a homogeneous system when focusing through the fused quartz of the cell cover. This cover is about 0.75 mm. thick, in order to insure its being made and maintained perfectly plane. Consequently, ordinary objectives of short focal length have too short working distance to focus through it, besides being corrected for much thinner cover-glasses. The immersion liquid should be kept clean and free from dust, and applied carefully to objective and to condenser in order to avoid bubbles which scatter light.

The adjustment of the illumination is carried out just as with an ordinary dark field illuminator (page 89). The light is centered on the aperture of the condenser, so as to supply a symmetrical cone; the trace of this cone may be observed as a circle of light on the top surface of the condenser. When the mirror is properly adjusted, the cell may be put in place, and examined with a low power objective or the naked eye to see whether the condenser is focused in the proper plane. It should be adjusted by raising or lowering the substage so that the tiny spot of light which marks the illuminated field shall be as small as possible. The glycerine immersion objective is next focused, without making any change in the illuminating system. If the illuminated area is off center, the objective (or the condenser) is centered, until particles over the entire field are uniformly bright. Unsymmetrical illumination may be recognized by noting whether the diffraction patterns are all one-sided, or whether they sway laterally on focusing. The utmost care in centration and in focusing both the objective and the condenser is necessary if a dark field and sharp diffraction patterns are to be obtained.

The cardioid ultramicroscope is practically restricted to the study of liquids and of plastic materials which can be pressed thin in the cell used. For coarse materials such as textile and paper fibers, or for any work requiring a moderately large field, the various types of ordinary dark field illuminators are preferable, and may be highly efficient if adequate illumination is supplied.

The Jentzsch Ultracondenser[12] can be placed upon the stage of any compound microscope and is so constructed as to combine in itself a reflecting condenser and cell for containing liquids, vapors or gases. It consists (Fig. 103) of a metal cell *M*, in which are mounted the two reflecting glass bodies *G*, *G′*. These are held in place by the cement *S*, *S*. Light rays enter the apparatus through the annular opening *O*, strike the silvered spherical surface in *G*, are reflected to the curved sides of *G′* — and enter the central cell *C*. The illuminating rays, therefore, are substantially at right angles to the optic axis of the microscope, thus conforming in general to those in the slit ultramicroscope with this difference, however, that in the slit instrument the rays enter the cell from one side only, whereas in the Jentzsch cell the rays enter from all sides and meet at the center.

[12] Made by Ernst Leitz, Wetzlar, and C. Baker, London.

A cover *N* fits into the mounting *M* and is secured in place by a bayonet catch. By turning the cover slightly it is made to press down upon the rubber gasket *RR*, making a very tight seal against the upper surface of *G′*. The tubes *TT* serve for the passage of gas or of liquid through the cell. The cover *N* is provided with a well-like depression closed at the end by the quartz plate *Q*. This well permits an objective of long working distance to be focused upon the particles in suspension at the focal point of the illuminating rays.

When in use the ultracondenser is laid upon the stage of the microscope with the short tube *A* inserted into the stage opening. The substage condenser is removed or swung aside. The plane mirror is then turned so as to reflect a beam of parallel rays into the device. This beam must be of such diameter as to fill the aperture of the condenser completely. A powerful source of light is essential, preferably an arc lamp or concentrated filament Mazda bulb. The mirror is tipped until the bright spot of light appears at the center of the cell. Since in this case we are examining the path of the rays as in the slit ultramicroscope and these rays enter from all sides and meet at the center, it is unnecessary to center the condenser exactly.

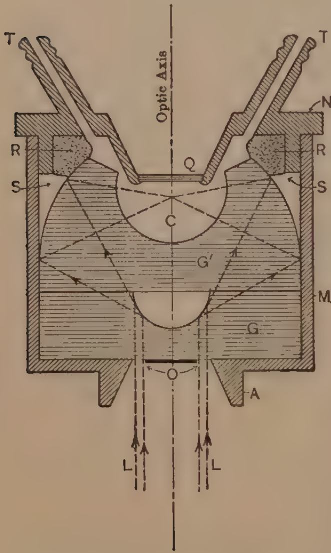

FIG. 103. Jentzsch Ultracondenser.

Special objectives of great penetrating power are necessary, corrected for the thickness of the quartz plate *Q* and with mountings of sufficiently small diameter to permit their entrance into the well in the cover to a depth such that the focal point will lie within the path of the rays. High magnifications must be obtained by employing high power eyepieces. It follows that there is always an illuminated plane lying below the focal plane of an objective and a perfectly black background is unobtainable. In order to obtain sharper contrasts, a diaphragm can be placed just above the mirror, either cutting off one side of the beam of light or having an opening slightly eccentric to that of the annular opening in the ultramicroscope.

The rubber gaskets are easily damaged by chemicals. The whole apparatus must be cleaned each time a different specimen is examined. For coarse colloidal suspensions in gases or water the ultracondenser is fairly satisfactory, but contrast is so poor that fine particles are not well defined, and the results are far inferior to those from other types of ultramicroscopes.

SPECIAL METHODS IN ULTRAMICROSCOPY[13]

Interpretations of Ultramicroscopic Appearances. — Since the "image" seen in the ultramicroscope does not necessarily duplicate the structure of the object, various indirect methods of interpreting it in terms of the properties of the latter have been developed.

Brownian Movement.[14] — Particles less than about 4 to 5 microns in diameter, suspended in fluid, show a continuous random dancing motion, when examined microscopically. This movement is ascribed to the impact of molecules in the suspending fluid; these molecules, though very much smaller than the particles, have high kinetic energy, and by more or less simultaneous impacts on one side or the other impart sufficient movement to the particles to be visible under the microscope.

The amplitude of the Brownian movement must be rather more than the resolving power of the microscope, if it is to be perceptible, and it must have sufficient duration to be noticed. Fine particles, of dimensions smaller than the limit of microscopic resolution, exhibit particularly lively motion under the ultramicroscope. The viscosity of the suspending fluid is also important; no Brownian movement is visible in glass or crystalline colloids, but particles in vapors and gases (smokes) show it most strikingly.[15]

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[13] Good discussions on ultramicroscopy are given in the following works:

Cotton and Mouton: *Les Ultramicroscopes et les Objets Ultramicroscopiques* (Hermann, Paris, 1906).

Heimstädt: *Apparate und Arbeitsmethoden der Ultramikroskopie und Dunkelfeld Beleuchtung* (Franckh'sche Verlag, Stuttgart, 1915).

Burton: *Physical Properties of Colloidal Solutions* (Longmans, Green & Co., London, 1921).

King: *Third British Association Report on Colloid Chemistry* (1920), pp. 31–41.

Zsigmondy: *Kolloidchemie* (O. Spamer, Leipzig, 1922).

Ostwald: *Licht und Farbe in Kolloiden*, I Teil. (Steinkopf, Leipzig, 1924), Chap. III, pp. 120–209.

Schirmann: Abderhalden's *Handbuch der biologischen Arbeitsmethoden.* Abt. II, Tl. 2, Hft. 1, pp. 774–816 (Urban & Schwarzenberg, Berlin, 1926).

Weigert: *Optische Methoden der Chemie* (Akademische Verlag, Leipzig, 1927).

von Hahn: *Dispersoidanalyse* (Steinkopf, Leipzig, 1928), pp. 401–24.

The reader is referred to the standard works on colloid chemistry for information regarding the general properties of colloids. The ultramicroscopic features of various special substances must be sought in the publications on the topics concerned.

[14] Brown: *Phil. Mag.* **4**, 101 (1826); **6**, 161 (1829).

Perrin: *Brownian Movement and Molecular Reality.* tr. by Soddy (1911).

Burton: *op. cit.* Chapter IV.

The increase in viscosity which accompanies the setting of gels has been shown by motion picture studies of the Brownian movement of mercury particles suspended in the gel.[16] Since the particles subject to the Brownian movement are moving in all directions, they may pass in or out of focus, with contraction and expansion of their diffraction patterns. A rotatory movement may result in a "twinkling" appearance, if the particles are non-spherical. Particles in motion are difficult to count or to study quantitatively.[17] If a shallow cell, such as that of the cardioid ultramicroscope, is used the particles may be allowed to condense upon its surfaces so as to be fixed in place in two planes; a film of stearin aids in anchoring them.[18] The kinetics of the Brownian movement and its influence on the settling of fine particles have been studied by many workers.[19]

Projection of the Brownian movement may be carried out as a demonstration, using a bispheric condenser,[20] a 10–30 ampere arc, and a preparation of high visibility.[21] "Titanox" pigment rubbed out in a dilute soap solution and decanted after settling a few minutes gives particles which exhibit brilliant diffraction patterns and lively movement. The room should be thoroughly dark, and the apparatus well screened so stray light cannot escape.

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[15] Zsigmondy: *Zeits. wiss. Mikros.* **24**, 104 (1907).

Lorenz and Eitel: *Zeits. anorg. Chem.* **87**, 357 (1914).

Gray, Speakman and Campbell: *Proc. Roy. Soc. Lond.* **102A**, 600, 615 (1923).

Regener: Freundlich's *Colloid and Capillary Chemistry* (1926), p. 779.

Gibbs: Alexander's *Colloid Chemistry* (1926), p. 393.

[16] Kraemer: *Jour. Phys. Chem.* **29**, 1523 (1925).

[17] Motion pictures have been taken through the ultramicroscope for this purpose by Kraemer: *Second Colloid Symposium Monograph* (1925), p. 57.

[18] van der Meulen and Reiman: *Jour. Amer. Chem. Soc.* **46**, 876 (1924).

[19] Perrin: *Comptes rendus* **146**, 967 (1908); **147**, 530 (1908); *Ann. de Chimie et de Physique* (8) **18**, 5 (1909); *loc. cit.*; Burton: Bogue's *Colloidal Behavior* (1924), Chap. V.; Freundlich: *Colloid and Capillary Chemistry* (1926), p. 341; Svedberg: *Colloid Chemistry* (1928), Part II; von Hahn: *op. cit.* pp. 349, 358.

[20] Leitz, or Zeiss "cardioid."

[21] Ehringhaus: *Kolloid Zeits.* **32**, 19 (1923) recommends a suspension of rutile. Black: *Jour. Chem. Educ.* **5**, 868 (1928) discusses in detail the technique of projection. See also Doane and Dow: *ibid.*, **6**, 1099 (1929).

Diffraction Patterns. — Although the phenomena of light seen with ultramicroscopic illumination do not constitute true microscopic images of the particles, they give considerable information as to their structure.[22] For a given intensity of illumination the brightness of the patterns varies with the difference between the refractive indices of the particle and the suspending medium. In the case of solvated colloids this may result in almost complete invisibility even though the particles are large. Ostwald[23] has suggested that monochromatic light be used, the wavelength selected being that for which the difference in refractive indices is greatest. However, such procedure is limited by the fact that strictly monochromatic light of high intensity is very difficult to secure. In general, the reflecting power of the particles is important, and metallic colloids usually have good visibility even though very fine. Although the size of ultramicroscopic particles is not directly represented by that of the diffraction pattern, large particles usually appear brighter than small ones of the same substance. Measurements of diffraction patterns have been used to determine the size of ultramicroscopic particles.[24]

If a particle is not substantially equidimensional it may not scatter light equally well in all directions. Platy or fibrous particles, as they are turned over by the Brownian movement, show fluctuations of brightness even when a single particle is kept in sharp focus.[25] Such particles are far from uncommon in colloids.[26] The non-spherical shape of colloidal particles is not generally evident with annular illumination, since the light comes from all sides. However, particles which have one dimension which is not ultramicroscopic will give evidence of their lack of symmetry. Unidirectional illumination is preferable for the study of such particles, and the slit ultramicroscope is most applicable for this work. By closing all but a part of the annular aperture of the cardioid ultramicroscope by means of an opaque screen, it may be used to furnish unilateral light. The " azimuth diaphragm "[27] has two openings opposite each other and adjustable in size, and gives bilateral illumination when used with the cardioid condenser.[28]

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[22] Ostwald: *Licht und Farbe in Kolloiden* (1924) deals in great detail with the various optical properties of colloids.

[23] *Kolloid Zeits.* **11**, 290 (1912).

[24] Gerhardt: *Zeits. für Physik.* **35**, 697 (1925); **44**, 397 (1927); *Ann. Physik.* **87**, 130, (1928); von Baeyer and Gerhardt: *idem.* **35**, 718 (1926); *Fortschritte der Chemie, Physik und physikalische Chemie.* **20B**. Hft. 1, pp. 1–23 (1928); von Hahn: *op. cit.* pp. 490–8.

[25] Detailed analyses of diffraction by particles of various shapes are given by Zsigmondy: *Zeits. wiss. Mikros.* **29**, 1 (1912); Schirmann: *loc. cit.*

[26] Freundlich: *Second Colloid Symposium Monograph* (1924), p. 46; *Berichte* **61B**. 2219–33 (1928); Zocher and Jacobson: *Kolloidchem. Beihefte.* **28**, 167–206 (1929).

Polarization of Light by Colloidal Particles. — Light diffracted by a particle does not have its plane of vibration materially changed, though its direction is altered. If a unidirectional beam of unpolarized light, with vibrations in all azimuths perpendicular to its direction of transmission, is scattered by a colloidal suspension, the scattered light will only possess vibrations in the plane normal to the axis of the beam. If the Tyndall cone of a slit ultramicroscope is observed from above, by means of an analyzing nicol prism, it will be practically completely " extinguished " if the plane of vibration of the nicol is adjusted parallel to the axis of the beam; the scattered light is polarized, and vibrates crosswise of the beam. If the Tyndall cone is observed from the side, by means of a nicol prism, its vibrations will be found to be in a vertical plane. If the illuminating beam is polarized, the Tyndall effect will be visible only from directions perpendicular to the plane of vibration of the beam. If the beam of light is polarized so as to vibrate in a vertical plane, it will be visible from the side, but not from above.[29]

Since the polarizing action of the particles is more or less nullified if the light comes from several directions, the slit ultramicroscope (or the cardioid, with azimuth diaphragm) is used for studying this property. A rotatable polarizing nicol prism may be placed in the path of the illuminating beam, or an analyzing nicol may be used above the eyepiece.

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[27] Siedentopf: *Zeits. wiss. Mikros.* **25**, 424 (1908); **29**, 1 (1912).

Szegvari: *Zeits. physik. Chem.* **112**, 277, 295 (1924); *Physik. Zeits.* **24**, 91 (1923).

Manufactured by Zeiss, and by Leitz.

[28] Ferric oxide sol, with platy particles, or benzopurpurin sol, with rod-shaped micellae, serve very well to demonstrate the above phenomena.

Freundlich: *Colloid and Capillary Chemistry* (1926), p. 407.

[29] A good discussion of the polarization of the Tyndall beam is given by Wood: *Physical Optics* (1919), pp. 625, 642; and by Freundlich: *Colloid and Capillary Chemistry* (1926), p. 379.

In dealing with very fine colloids, oils, solutions of dyes, etc., in which only the Tyndall beam is seen, it is necessary to determine whether the scattering of light is due to diffraction or to fluorescence. In general, fluorescent light in isotropic media such as liquids is practically unpolarized.[30]

The polarizing properties of suspensions of non-spherical particles are not particularly out of the ordinary, as long as the particles are in purely random orientation. If some force, such as tension, compression or shear, or an electric or magnetic field, is applied to the suspension, the arrangement of the particles tends to become more or less systematic and parallel. Currents in liquid colloids cause marked alignment of the particles, and enhanced polarizing properties in certain directions. This "streaming anisotropy"[31] can be demonstrated under the ultramicroscope, or even to the naked eye, by inducing a rotary or continuous flow in a suspension of platy or rod-like particles. If ordinary light is used there is simply a greater scattering of light in certain directions and less in others. Between crossed nicol prisms such oriented suspensions show distinct double refraction which is closely related to the double refraction sometimes exhibited by other types of colloidal aggregates such as gels (see page 303). Zocher[32] has discussed the characteristics of various types of anisotropy in colloids, in considerable detail.

Color Phenomena. — Most colloidal systems exhibit coloring which is not obviously related to the color of the material in mass.[33] This may range from the blue light which is scattered by relatively coarse particles of fairly colorless material, to highly complicated phenomena in the case of selectively reflecting substances such as metals. In general it may be said that the color of the scattered light is approximately complementary to that of the transmitted light. Transparent particles much coarser than 1 μ[34] scatter white light. If they are rather less than 0.5 μ in diameter, the scattered light is blue, and the transmitted light orange or reddish. Transparent particles much finer than this show little difference between the color of the scattered and of the transmitted light, unless they possess strong reflecting power, as do colloidal metals or dyestuffs. These classes of substances frequently exhibit striking color effects, as in the case of the well-known gold ruby glass, which is red by transmitted light and scatters green light. Many attempts have been made to formulate a theoretical explanation which will cover all cases of color from colloidal particles, but so far no great generalizations can be made. The variations in color which a single substance may manifest are usually considered to be due either to different degrees of fineness of its particles or to differences in the aggregation of these particles. Undoubtedly the conditions of formation and the nature of the peptizing agent and of the suspending medium have a marked influence in many instances.

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[30] Not wholly, however. See Weigert: *Verh. deutsch. physik. Ges.* (3) **1**, 100 (1920).

[31] Freundlich, Stapelfeldt, and Zocher: *Zeits. Physik. Chem.* **114**, 161–207 (1924).

Freundlich: *Colloid and Capillary Chemistry* (1926), p. 403.

Ambronn and Frey: *Das Polarisationsmikroskop* (Leipzig, 1926), p. 161.

[32] Freundlich, *op. cit.*, p. 720; *Koll. Zeits.* **37**, 336 (1925).

[33] See the various references already cited, and also Bancroft's series of papers on The Colors of Colloids, *Jour. Phys. Chem.* **22**, **23** (1918–1919).

In the case of non-spherical colloidal particles, the colors may vary markedly depending on the orientation and alignment of the micellae. This may manifest itself in pleochroism in the sol or gel when some external force is applied.

Form and Size of Ultramicroscopic Particles. — The **form** of colloidal particles, if all dimensions are beyond the limit of resolution, is determined from the evidence which has already been discussed: " twinkling," streaming anisotropy, double refraction and pleochroism, and other properties dependent on the non-spherical shape of the particles. Most metals seem to give approximately spherical particles; oxides may give plates or needles, as may certain dyestuffs and other organic colloids. Freundlich[35] tabulates the behavior of the Tyndall light with rods and plates in flowing liquids, as a basis for distinguishing which type of particles is present. In general, the coarser the particles the better defined are the characteristic properties.

The **size** of ultramicroscopic particles may be ascertained by a number of methods,[36] microscopical and otherwise. Of the microscopical methods the original ones of Siedentopf and Zsigmondy[37] are the most generally applicable. Either the number of particles in a unit volume (*cf.* page 417) or the average distance between the particles is the basis of the determination.

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[34] 1 μ (micron) = 0.001 mm.
1 m μ (millimicron) = 0.001 μ.
The micron is the unit of small measurements, and is used widely in microscopy and colloid chemistry.

[35] *op. cit.*, p. 406.

The number of particles in unit volume is determined by direct count.[38] In order to do this, it is necessary that the individual particles shall be clearly visible, that only a small number shall be in the field, and that the volume examined shall be sharply defined. If the preparation is too concentrated, it must be diluted, and this must be taken into account in the subsequent calculations. With the slit ultramicroscope the volume of solution studied is defined by the illuminating beam. The thickness of this beam may be decreased if the particles are closely superposed; it is then turned edgewise by rotating the slit, and measured by a calibrated eyepiece micrometer in the microscope.[39] The depth of the volume in which particles are counted is thus determined. The cross-ruled area of the micrometer measures the length and width of this volume, so its cubic capacity can be readily calculated. The particles in this volume are counted, and the preparation is moved about so that counts may be taken on different fields. The Brownian movement of the particles may render the determinations far from uniform, and a large number of readings should be made to insure consistent results. Assuming the particles to be spheres, their average radius is represented by the formula

$$r = \sqrt[3]{\frac{3\,M}{4\,\pi\rho n}}$$

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[36] Svedberg: *Colloid Chemistry* (1928), pp. 128–201.
Burton: *Physical Properties of Colloidal Solutions* (1921), Chap. VI; Alexander's *Colloid Chemistry* (1926), p. 165.
Kuhn: *Koll. Zeits.* **37**, 365 (1925).
Tendeloo: *Chem. Weekblad* **25**, 158 (1928).
von Hahn: *Dispersoidanalyse* (Leipzig, 1928).

[37] *Verh. der deutsch physik. Ges.* **5**, 209 (1903); *Ann. der Physik* (4) **10**, 16 (1903).

[38] Weigener: *Koll. Chem. Beihefte.* **2**, 213 (1911), discusses the accuracy of the counting operation.

[39] The method of calibration is given on page 398.

where M represents the total mass in the volume counted (as determined from analytical data), ρ the density, and n the number of particles. Such a determination is based upon a number of questionable but rather unavoidable assumptions: that the particles are spheres, that their density is the same as that of the material in mass, that all the material is present in the form of visible particles and none of it in true solution, that no change in the degree of dispersion or size of particles has taken place on dilution, and that the particles are uniform in size. Whether these postulates are correct or not must be decided by other, non-microscopical, methods.

The cardioid ultramicroscope may be used instead of the slit ultramicroscope, by applying the above principles. The volume of solution in which particles are counted is defined by the thickness of the layer of liquid in the cell, as measured by the fine adjustment of the microscope, and by the area of the field included in the rulings of the eyepiece micrometer. The cover of the cell must be pressed down tightly each time, to be sure that the thickness of layer is constant. A haemacytometer cell (page 435) may be used to contain the liquid, if a thicker layer is desired, since the distance between slide and cover-glass is 0.1 mm. in this case. To count all the particles, it is necessary to shift the focus of the objective, and the suspension should not be too concentrated.[40] In either of these shallow cells, the particles may be allowed to come to rest upon the top and bottom surfaces before counting, if the Brownian movement is troublesome.[41] Colloidal particles in plastic material, such as carbon black in rubber, may be pressed into a thin film and counted as above.

The second of the methods proposed by Siedentopf and Zsigmondy[42] depends on the estimation of the average distance between the particles in the colloidal suspension under examination. The average particle size is given by the formula

$$r = \sqrt[3]{\frac{M}{\rho} \cdot d}$$

where d is the mean distance between particles. If the Brownian movement is lively, this distance is difficult to estimate, and counting methods are rather more simple, in general.

[40] Burton: *Phil. Mag.* (6) **11**, 425 (1906).

[41] Perrin: *Bull. Soc. Fr. Phys.* **3**, 155 (1909).

[42] *Loc. cit.*

Other microscopical methods of determining the size of ultramicroscopic particles are based on the rate of settling or of migration in an electric field;[43] and the shift, by diffusion, of the boundary between a colloidal solution and another liquid, as measured in a cell under the microscope.[44] Measurements of the amplitude of the Brownian movement have also been used for this purpose.[45]

Particle-size determinations in colloidal suspensions are valid only at the time they are made and at the particular dilution required. Various factors may cause an alteration in the size of the particles, the growth of new ones, or the agglomeration of particles into aggregates. If the aggregates are not too dense, the individual particles in them may sometimes be counted.

The average particle-size given by the above methods does not reveal the uniformity of size, and various procedures have been devised to furnish this information.[46] If the particles are coarse enough to settle out on standing, individuals may be followed under the microscope, and their rate of fall measured.[47] Calculations using Stoke's law and based on a large number of particles will yield a size-frequency curve.[48] With finer particles, centrifugal force has been employed to accelerate settling,[49] and to sort out the particles of different sizes.

Electrical Properties of Ultramicroscopic Particles. — Colloidal particles own many of their properties to the electrical charges which they bear;[50] the sign of the charge may be determined under the ultramicroscope by a number of direct methods. These are all based on the migration (cataphoresis) of the particles through the suspending medium, when an electromotive force is applied. This movement is complicated by the fact that currents in the solution are set up along the surfaces of the cell in which the observation is made (electrical endosmose) and a reverse migration occurs. This must either be taken into account, according to v. Smoluchowski's formula,[51] or else the cell must be designed so that its effect is of definite magnitude.[52] The true cataphoretic mobility is observed in a flat cell at distances from the walls of approximately $\frac{1}{5}$ or $\frac{4}{5}$ the thickness of the layer. Measurements by means of the fine adjustment of the microscope are necessary to insure that only particles in these zones shall be in focus.

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[43] Wells and Gerke: *Jour. Amer. Chem. Soc.* **41**, 312 (1919).
Rohmann: *Zeits. Physik.* **17**, 253 (1923).
Blüh: *Koll. Zeits.* **37**, 267 (1925).
von Hahn: *op. cit.*, p. 346.

[44] Fürth: *Koll. Zeits.* **41**, 300 (1927).
Fürth and Ullman: *ibid.* **41**, 304 (1927).

[45] See the references given on page 228.

[46] Svedberg: *loc. cit.*

[47] Chakravarti and Dhar: *Kolloid Zeits*, **44**, 63 (1928).

[48] Svedberg and Estrup: *Koll. Zeits.* **9**, 259 (1911).
Wells and Gerke: *loc. cit.*
See also page 414.

[49] Westgren: *Zeits. anorg. Chem.* **94**, 193 (1916).

[50] Michaelis: Alexander's *Colloid Chemistry* (1926), p. 471.

The potential applied varies over wide limits, depending on the conductivity of the suspending liquid. In general the current passed should be small, to avoid polarization or liberation of gas at the electrodes. The electrodes may be kept from actual contact with the colloidal suspension being studied, by means of a communicating chamber filled with a depolarizing solution of similar concentration. The velocity of cataphoresis is dependent on the size and shape of the particle, and is also governed by the potential of its electrical double layer, which may be determined from quantitative measurements of the rate of migration in a given electric field.[53]

Since the early work of Quincke,[54] who discovered the reverse flow near the walls of the container, many investigators have studied cataphoresis by means of the microscope. Depending on the type of ultramicroscopic illumination, various different kinds of cells have been used. With the cardioid or other reflecting condensers, the specimen is contained in a shallow cell on an ordinary microscope slide, at the ends of which are the electrodes.[55] Of these the simplest is that described by Svedberg,[56] which consists of a microscope slide, across which two strips of platinum foil are cemented 1 or 2 cm. apart, by means of de Khotinsky cement. Their thickness determines the depth of the cell, about 0.01 mm., and on them is cemented a cover-glass. The sides are left open, and after filling are closed by vaseline or wax. Polarization at the electrodes is a disturbing factor: its influence may be minimized by very short periods of electrolysis, or by the use of alternating current.

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[51] Michaelis: *loc. cit.*, p. 476.
Graetz: *Handb. der Elektrizität* (1914) **2**, 382.

[52] Mooney: *Phys. Rev.* **29**, 218 (1927).
Falk, Jensen, and Mills: *Jour. Bacteriol.* **15**, 440 (1928).

[53] von Hahn: *op. cit.*, p. 346.

[54] *Ann. physik. Chem.* (2) **113**, 513.

[55] Cotton and Mouton: *Comptes rendus* **138**, 1584, 1692 (1904); *Jour. chim. physique* **4**, 365 (1906); *Les Ultramicroscopes* (1906), Chap. VII.
Ellis: *Zeits. physik. Chem.* **78**, 321 (1911).
Masamichi Kimura: *Chem. Zentr.* **1**, 96 (1914).
Powis: *Zeits. physik. Chem.* **89**, 91 (1915).

If the slit ultramicroscope is used, a deeper cell is possible, but the effect of reverse currents is not eliminated. Electrodes may be inserted in cells of the Biltz type, but that of Northrop and Kunitz is more useful.[57] It consists of a glass tube, flattened to approximately rectangular cross-section, communicating with two chambers containing zinc electrodes in zinc sulphate solution, which prevent polarization effects. The cell can be placed on the stage of a slit ultramicroscope; orthogonal illumination enters it edgewise, and the particles are viewed through the flat surface by means of the microscope. Two platinum wires are sealed transversely 2 cm. apart in the flattened portion of the cell, for determination of the fall of potential in the solution. The apparatus can be constructed by any competent glass blower, and by the use of standard interchangeable ground joints the central portion may be readily removed for cleaning, or replaced if broken.[58] The influence of endosmotic currents must be guarded against, just as in the cells of flat type.

Precipitation and Peptization. — The well-known phenomena of flocculation by electrolytes or by colloids of opposite charge, the action of diluents or of peptizing agents, and the influence of

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Svedberg and Andersson: *Koll. Zeits.* **24**, 156 (1919).
Freundlich and Abramson: *Zeits. physik. Chem.* **128**, 26 (1927).
Tourila: *Kolloid Zeits.* **44**, 11 (1928).
Falk, Jensen, and Mills: *Jour. Bacteriol.* **15**, 421–50 (1928).
Kruyt and v. d. Willigen: *Kolloid Zeits.* **44**, 22 (1928).
Mattson: *Jour. Phys. Chem.* **32**, 1532 (1928).
Tiselius: Abderhalden's *Handbuch der biologischen Arbeitsmethoden*, Abt. III, Teil B, Heft. 4, pp. 669–675.

[56] *Loc. cit.*; *Colloid Chemistry* (1928), p. 233.

[57] *Jour. Gen. Physiol.* **4**, 629 (1921–2); **6**, 413 (1923–4); **7**, 729 (1925). Obtainable from Eimer & Amend, New York.

[58] A modification of the apparatus, as described by Northrop and Kunitz, was utilized by Abramson: *Sixth Colloid Symposium Monograph* (1928), p. 115.

light, heat or various chemical reagents on colloidal suspensions may be demonstrated under the ultramicroscope. Any of the cells described may be used; a perforated cover-glass, such as that supplied with the cardioid condenser, permits the convenient addition of reagents to the specimen while it is under observation.

Aggregates and Adsorbing Surfaces. — The size and density of aggregates obtained by coagulating a colloidal suspension are easily studied ultramicroscopically. Unstable or partially coagulated sols show characteristic appearances. Precipitation of colloidal particles on surfaces such as those of textile or paper fibers is worthy of study in connection with dyeing, mordanting, sizing or detergent operations. Aggregates of micellae such as constitute cellulose or silk fibers may profitably be studied by means of the ultramicroscope,[59] as may the various stages in the gelatinization and coagulation of cellulose to form rayons.

In general, little information as to the actual adsorbing surface presented by solid colloids can be gained from microscopic examinations. The finer pores of boneblack, silica gel, coconut charcoal, and similar adsorbents are of ultramicroscopic dimensions, and are too close to each other to be resolved, even if it were possible to prepare a specimen thin enough to include a single layer of pores. Examination of the external surface of such adsorbents may be of value, however, as an indication of its freedom from any glaze or other clogging layer.

Limits of Ultramicroscopic Observation. — No definite limits to the range of ultramicroscopic visibility can be fixed. The nature of the particles is the chief factor, the illumination being equal. By means of the immersion ultramicroscope and direct sunlight, Zsigmondy observed particles of colloidal gold about 1 m μ in diameter; with the slit ultramicroscope and an arc lamp, 5–15 m μ is about the limit. Colloidal particles of less diffracting power are only visible when many times coarser than this.[60] It is possible to render visible particles which are beyond the limit of the ultramicroscope, by depositing gold upon them so as to increase their diffracting power,[61] though this procedure is subject to errors which may not be readily detected.

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[59] Herzog: *Mikroskopische Untersuchung der Seide und Kunstseide* (Berlin, 1924), p. 83.

[60] Freundlich: *Colloid and Capillary Chemistry* (1926), p. 562.

[61] Zsigmondy: *Zeits. physik. Chem.* **56**, 65, 77 (1906).
Bechhold and Villa: *Biochem. Zeits.* **165**, 250 (1925).

CHAPTER VIII

PHOTOMICROGRAPHY AND MICROPROJECTION

By utilizing the microscope to form an image on a photographic plate or on a screen, its advantages in the examination of the structure and properties of minute objects are greatly extended, and are made available to others besides the person manipulating the instrument. Anyone, however inexperienced in the use of the microscope or lacking in the skill necessary to prepare material for study, can view the photographed or projected image, and can see all that is there depicted. However, the observer is wholly dependent upon the skill of the microscopist who produced the image, and can see only what is put before him. To him, this image is the basis of his entire concept of the nature of the object, and, in the attempt to extract from it the fullest interpretation, he is likely to credit it with an unmerited validity. To the microscopist, this image is only one of many aspects of the specimen, any of which is at his disposal by a shift of the field, a touch of the fine adjustment, or a variation in the character of the illumination.

In a record or a report, properly prepared photomicrographs accompanied by a brief comment can take the place of pages of discussion of appearances which are impossible to represent by words alone. Similarly, a projected image of a microscopic object can save a great deal of more or less vague description, and insures that the same concrete idea is conveyed to the different observers. The photomicrograph has the advantages of convenience and permanency, but at best can represent only a selected view of the object. The photographic print or the image on the screen may be seen by many persons simultaneously, but this passive observation is far less instructive or fruitful than an examination of the object at first hand.

In either case it is first necessary for the microscopist to form a concept of the nature of the specimen, as a result of detailed study by various methods. Only then may he be justified in choosing a particular appearance to record or to exhibit to others as most truly representative of its actual character. And only by virtue of the technique and knowledge which he must acquire and use in these preliminary studies, can he hope to produce an image which will adequately display the features which he deems significant. An intelligent decision as to *what* is to be shown, and careful experimentation as to *how* best to show it are essential to successful photomicrography and microprojection.

A great many types of objects may be photographed or projected under almost standardized conditions, and the results will be comparable and useful. It is chiefly in the case of highly critical studies, of examinations of novel materials, or of search for unfamiliar properties, that these methods are of limited application and must be subject to criticism or confirmation by visual investigations.

However perfect the technique, there will probably always be some objects which are better rendered in a drawing than in a projected image. Careful drawings can integrate a whole series of appearances of the same object at different positions of focus, and can emphasize important features or suppress extraneous material, to the improvement of the final picture. As regards accuracy of rendition, even a moderately skilled draftsman can record a static image directly, while by means of a drawing camera (page 395) or a projection drawing apparatus (page 263), the dimensions and proportions of large and complex specimens may be accurately reproduced to scale. Drawings are particularly valuable as an aid in interpreting appearances, for each line must represent a given structure, and this necessitates a rigorous analysis of the image, detail by detail, when it is being put down on paper. There is no better training for the observational powers of a microscopist than making accurate and concise drawings, in which the features represented depend first upon his own ability to see them, rather than on a semi-automatic process of photographic reproduction. Since no method of reproduction of microscopic appearances can eliminate the personal equation, it is highly desirable that too much faith should not be put in routine procedures, and that detailed and painstaking visual examination should be the basis of either graphic or projection methods.[1]

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[1] An excellent discussion of the advantages and disadvantages of drawings and photomicrographs is given by Lawrie: *Textile Microscopy* (London, 1928), Chap. VI. See also Gage: *The Microscope* (1925), Chap. VI, and Wallis: *Analytical Microscopy* (London, 1923), p. 117.

A number of books are available, on photomicrography and on microprojection.[2] Besides these, most of the books on metallography discuss the photomicrography of opaque objects.[3] Numerous articles have also been published in the various journals, which deal with special applications and methods of photomicrography. Only a few of these will be referred to in the present chapter.[4]

The optical systems used in photomicrography and microprojection are closely similar, as are the methods of illumination. Their intelligent use necessitates a thorough understanding of the principles of direct visual microscopy, since the quality of the projected image is governed by the same laws and subject to the same limitations. In general it may be said that one should not be content with an image on the ground-glass of the camera or the projection screen which is less perfect than what he would demand in visual observation. The obtaining of such an image is largely a matter of microscopical technique; knowledge of the special procedures and equipment peculiar to photomicrography and projection may be acquired without great difficulty if such a foundation is available. The present chapter deals only with apparatus and methods which are not covered in the chapters on the microscope and its illuminating systems.

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[2] Hind and Randles: *Handbook of Photomicrography* (G. Routledge & Sons, London, 1927).

Barnard and Welch: *Practical Photomicrography* (Longmans, Green & Co., London, 1925).

S. H. and H. P. Gage: *Optic Projection* (Comstock Pub. Co., Ithaca, N. Y., 1914).

Köhler: *Mikrophotographie*, in Abderhalden's *Handbuch der biologischen Arbeitsmethoden* Abt. II, Tl. 2, Hft. 6, pp. 1691–1976 (Urban & Schwarzenberg, Berlin, 1927).

Monypenny: Photography in the Engineering and Metallurgical Industries, Chap. VII of *Photography as a Scientific Implement* (Van Nostrand, New York, 1923).

Rodman: Photomicrography. Chap. VII, *ibid.*

Eastman Kodak Co.: *Photomicrography* (1927).

Krause: *Encyclopädie der Mikroskopischen Technik*, II Band (Berlin, 1926), pp. 1450–88.

Metzner: *Das Mikroskop* (1928), Chap. VIII.

Loveland and Trivelli: Practical Photomicrography — Chap. IV of Fairbanks' *Laboratory Investigation* of Ores (McGraw-Hill Book Co., New York, 1928).

Gage: *The Microscope* (1925), Chaps. VI and VII.

Laubenheimer: *Lehrbuch der Mikrophotographie* (Berlin, 1920).

Neuhauss: *Lehrbuch der Mikrophotographie* (Leipzig, 1907).

[3] See the references cited on p. 112.

[4] Lofton: Photomicrography of Paper Fibers, *Techn. Paper.* Bur. Stds. **217.**

Wightman and Trivelli: Photography through Microscope, *Amer. Annual of Photog.* **1928.**

Lucas: Photomicrography and Its Application to Mechanical Engineering, *Mech. Eng.* **50**, 205 (1928).
Photomicrography and Technical Microscopy, *Bell System Techn. Jour.* **3**, 100–144 (1924).
High Power Photomicrography of Metallurgical Specimens, *Trans. Amer. Soc. Steel Treat.* **4**, 613 (1923).

Hanna: *Trans. Amer. Micros. Soc.* **46**, 15–25 (1927).

The image which is obtained on the photographic plate or on the projection screen is a real image. It may be formed in either of two ways:

1 — By the objective, without an eyepiece.
2 — By the objective and eyepiece acting jointly.

Projection of images by the objective alone is a method ordinarily used only for low magnifications (5 to 50×) where large fields have to be included and where depth of focus, rather than resolution, is desired. It is particularly appropriate to the study of coarsely ground material, fractures in test pieces, small manufactured articles, and the effects of corrosion or abrasion. The relationships of various parts of the specimen under examination can be clearly brought out, and the location from which a sample was taken for detailed investigation at higher magnification is easily shown. It frequently happens in chemical or metallographic work that the gross structure of specimens has to be recorded, and a knowledge of low-power photomicrography is widely useful in dealing with macroscopic as well as microscopic objects.

The objective is placed so as to form an enlarged real image, the size of which is determined by the relative distances from the lens of the image and object (page 5). Increase in magnification is accomplished by increasing the projection distance, or by using an objective of shorter focal length. In photomicrography, projection distances are usually less than a meter (the length of the ordinary camera bellows when fully extended). Low-power objectives (32-mm., 48-mm.) of the ordinary type may be used; but since these lenses are designed to project a real image at a short distance, and to cover a small field, they are not as suitable as specially corrected low-power **photographic objectives.**[5] The focal lengths obtainable range from 16 mm. to 150 mm.; the numerical apertures are relatively low (0.10–0.15). Photographic objectives are particularly well corrected for astigmatism, curvature of field, and distortion, because these defects are especially prominent if large fields are imaged. An iris diaphragm, either as an integral part of the objective or attached just above it, is useful for increasing the depth of focus in the case of irregular objects. In order that the upper end of the draw-tube shall not restrict the area of the projected image, such lenses should be used on a stand equipped with a wide body-tube, the top and draw-tube of which can be readily removed. The lowest powers are sometimes mounted in an adapter directly on the front of the camera, though this renders focusing less convenient.

Illumination by transmitted light is effected by a condenser of the " spectacle lens " type. A ground-glass screen in front of the light source is an aid in securing even illumination. White paper or opal glass may be placed at an angle below the specimen, to diffuse light upward over a large area, although it results in lessened intensity, and is not feasible for projection on a viewing screen.

Opaque objects may be illuminated either by a direct beam from a lamp and auxiliary condenser, or by means of a transparent reflector placed in front of the objective (Fig. 47) if the working distance permits. A ground-glass diffusing screen may be placed in the path of the light, in order to give more uniform illumination over a larger area or to throw some light into the shadows of the specimen. Inclined illumination from more than one direction is particularly useful for the latter purpose, and helps to give a three-dimensional appearance to the image.

The larger metallographic microscopes may be equipped with accessory apparatus to permit the photography of large and irregular objects. Some form of auxiliary stage, capable of bearing a heavy specimen, is desirable. A ball-and-socket support,[6] preferably one which can be raised or lowered by means of a rack-and-pinion, is invaluable as an aid in low-power photography without a microscope stand; it permits the object to be tilted in any direction and considerably facilitates focusing.

[5] All manufacturers list a series of lenses of this type.

[6] Obtainable from Leitz or Zeiss.

If transmitted illumination is desired at low magnifications, a plate of glass, supported on legs, makes a useful stage of large area, on which may be placed crystallizing dishes, reaction tubes, and similar apparatus. A large mirror or a sheet of paper beneath it will give satisfactory diffuse light.

Projection of images by the objective and eyepiece enables higher magnifications to be obtained than are possible with the objective alone. Furthermore, the objective forms its real image at the normal tube length, so that greater resolution and better corrections are possible. Although the field is not as large as if no eyepiece were used, there is a gain in manipulative convenience and compactness of the apparatus.

The objectives and eyepieces employed are of the ordinary types, and are mounted on a stand as in visual work. The focus is adjusted so that the objective forms a real image (F_2, Fig. 104) *outside* the focal plane of the eyepiece, in such a position that this image is projected on the photographic plate or viewing screen by the eye lens. The final image F_3 is thus reinverted, or erect. Its size is governed by the factors given on page 7, and the projection distance. The formula for the approximate magnification of the compound microscope (**4**, page 7) is applicable if the projected image is formed at a distance of 250 mm. from the eyepoint. At greater or less distances the magnification is directly proportional to the projection distance:

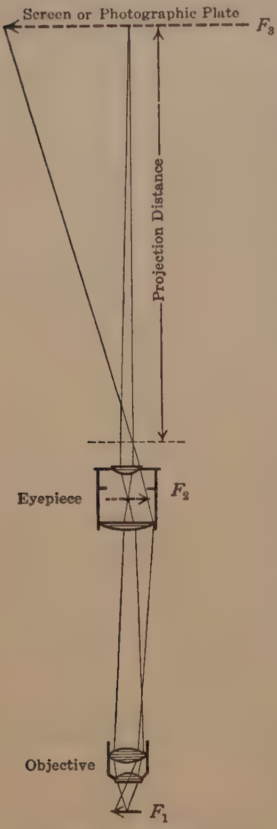

FIG. 104. Projection of a Real Image by Objective and Eyepiece.

$$\text{image magnification} = \text{magnification of microscope} \times \frac{\text{projection distance}}{250 \text{ mm.}}$$

Variation of the magnification of the system may be accomplished by changing any of the following: focal length of objective, tube length, focal length of eyepiece, projection distance.

The **lens system** used in microprojection need not be of exceptional quality, since the image is examined visually, usually from some distance away, and details are likely to be lost. In photomicrography, however, the optical system should be of the highest quality if resolution is sought. Apochromatic objectives are desirable, for they may be focused visually and they will be equally in focus for the actinic portion of the light. Achromatic objectives, when used with approximately monochromatic green light from a suitable color filter, are fully as satisfactory in this respect but may not have quite such good general corrections. The achromats are occasionally superior as regards flatness of field and may be preferred in metallography.

Penetrating power is particularly desirable in photomicrography, especially if the specimen possesses appreciable depth, for a photographic negative records only what is in focus when the exposure is made. It is frequently necessary to sacrifice resolving power in order to show a reasonable amount of the third dimension of the object. For such purposes an objective with iris diaphragm (page 16) is invaluable since its depth of focus may be increased just enough to show the entire structure of the object or to " flatten " the field, with no more loss of resolution than is absolutely unavoidable. It is much more convenient to make this adjustment for the particular field chosen than to be forced to change to another objective of lower numerical aperture and to rearrange the whole system accordingly.

If well corrected objectives are used for photomicrography, the tube length of the microscope is important. Instead of forming the real image in the lower focal plane of the eyepiece, the objective must be focused to form it at a less distance, unless the draw-tube is extended. With low-powered eyepieces and short projection distances a decrease in flatness of field may result. Patterson[7] gives a formula for the amount of withdrawal of the tube or eyepiece:

$$\frac{\text{square of focal length of eyepiece}}{\text{projection distance}}.$$

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[7] *The Optics of Metallography,* Appendix to Sauveur's *Metallography.* Reprinted by Bausch & Lomb. Similar information is tabulated by Metzner: *Das Mikroskop,* p. 425.

Projection eyepieces, when of low power, are specially corrected for this reason and are often provided with a movable eye lens. This movable lens is shifted until the field diaphragm in the eyepiece is projected sharply. The objective is then focused and forms its image in the plane of this diaphragm, at the normal tube length.

Compensating or semi-compensating flat-field eyepieces are essential if a considerable share of the magnification is effected by the eyepiece. They vary markedly in flatness of field and distortion, and should be selected after trial. In general, the projection distance used should be greater than 250 mm. if an image of good quality is to be obtained. Amplifying lenses (Fig. 13) in place of eyepieces give excellent chromatic correction and flat fields. They are not designed for excessively long or short projection distances.

The **illuminating system** for photomicrography and microprojection should be essentially the same as that used in visual work. In general, more brilliant illumination is desirable, and particular effort should be made to secure contrast and sharpness of outline. A substage condenser is desirable, even for low powers, and it should be practically in focus on the object. For high powers, critical illumination by Köhler's method (page 98) affords the best control of field and aperture. Vertical illuminators of the mirror or prism reflector type give brighter and more contrasty images than those with transparent reflectors, and should be used unless resolution is particularly sought. Dark field illumination is not generally adequate for microprojection on account of the small amount of light actually scattered by the particles, though good results may be obtained with a preparation of high visibility and a particularly powerful light source.[8] Photography by dark field or ultramicroscopic illumination is feasible if a long exposure is given; if "fast" plates are used, instantaneous exposures may be employed.

Auxiliary condensers are practically indispensable for projection or photomicrography at moderate or high magnifications. Their arrangement, and the position of field and aperture diaphragms, is discussed in Chapters III and IV. The illuminating system of the large metallographic microscopes is of course particularly designed for photographic use.

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[8] See page 228.

Light sources of high intensity are of the utmost importance in microprojection, since a bright image several feet in diameter must be thrown on the screen, and must be clearly visible even in a poorly darkened room. An arc lamp of 8 or more amperes is barely sufficient, especially if projection with polarized light or dark field illumination is to be undertaken. The proper use of well corrected auxiliary condensers, to give the maximum concentration of light, is necessary under these conditions. The size and bulkiness of these condensers is not a measure of their efficiency, however, and a small condenser placed close to the arc may have greater light-grasping and light-transmitting power than a much larger lens poorly located.

Photomicrography does not demand such brilliant illumination, except for very high magnifications. Incandescent lamps with "projection model" filaments (page 105) are adequate for most work. They should be used behind a ground-glass screen, if ordinary critical illumination is attempted, so that the filament will not be imaged in the plane of the object. If Köhler's method of illumination is used this precaution is not necessary, since the filament is imaged in the aperture of the substage condenser. Either the tungsten arc or the ribbon filament lamp supplies a homogeneous light source which is excellent for photomicrographic work at any but the highest magnifications. The carbon arc is of course much more intense and actinic and shortens the time of exposure greatly — frequently an important factor if vibration is serious or the preparation is likely to change. The use of color filters in photomicrography is discussed on page 101.

If an arc lamp is used for microprojection or photomicrography it should be equipped with a reliable automatic clockwork feed, so that the worker need give it a minimum of attention and so that its brightness and position will remain practically constant. Particularly in microprojection before an audience, and in the painstaking focusing which is necessary for successful photomicrography at high powers, the microscopist should be able to give his whole attention to the image which is on the focusing screen.

It will usually be found that the proper **adjustment of the illumination** is the most exacting part of the manipulation, in either photomicrography or projection. For this reason it is desirable that all auxiliary condensers and diaphragms be mounted on an optical bench, such as is an essential feature of the outfits supplied by most makers. The light source must be imaged to fill the aperture of the substage condenser uniformly, or unsymmetrical shading and swaying of the image on focusing will result. The aperture diaphragm of the condenser must be manipulated with the utmost care, in order to give well contrasted outlines of refraction images without loss of resolution or brilliancy, and without the development of diffraction haloes at each boundary surface. Some time may well be spent in trying different degrees of convergence of light, and in testing the effect of unsymmetrical illumination of various degrees of obliquity and from different azimuths. An excenterable substage diaphragm is very convenient for such work, for it permits the illumination to be maintained or duplicated once a satisfactory adjustment has been accomplished. The field must be uniformly lighted, and this is particularly difficult to achieve because the eye fails to notice variations which later manifest themselves on the photographic plate. To judge the uniformity of illumination the eye should be held exactly over the center of the ground-glass focusing screen of the camera, at a distance of at least a foot from it. The mirror may then be manipulated to give an evenly bright field.

APPARATUS AND METHODS OF PHOTOMICROGRAPHY

The simplest apparatus for photomicrography consists of an ordinary microscope, a light source, and a camera. In most chemical work, particularly if liquid preparations are being studied, a vertical position of the microscope is desirable.

"Photographic eyepieces." — A number of small cameras are on the market, which are attachable to any microscope for more or less impromptu photomicrography.

Of these, the authors prefer the "Makam" of Leitz (Fig. 105). This instrument consists of an eyepiece, and a camera of fixed extension. Just above the lenses of the eyepieces are mounted a shutter and a removable semi-transparent reflector which diverts about 25 per cent of the light to the observation tube. By looking in this tube the microscope can be focused while the plate is in place, and during the exposure if necessary. A rotary adjustment provides for coincidence of visual and photographic foci. The magnification is fixed, and is practically equivalent to that of a 10× eyepiece at 250-mm. projection distance.

The instrument is light in weight and should cause no shift of focus if the coarse adjustment of the microscope is not unduly free-working. The shutter permits time exposures, or instantaneous exposures up to one second. Unfortunately, the plate holders require adapters to fit them for $3\frac{1}{4} \times 4\frac{1}{4}$-inch plates. A film pack may be used if desired. The smaller model "Macca" camera has about half the magnifying power, and uses $1\frac{3}{4} \times 2\frac{3}{8}$-inch plates. Zeiss manufactures a photographic eyepiece, "Phoku," in which is incorporated a lens similar to the Homal, but this instrument uses very small plates (6 × 4.5 cm.). The Bausch & Lomb "Type K" Camera

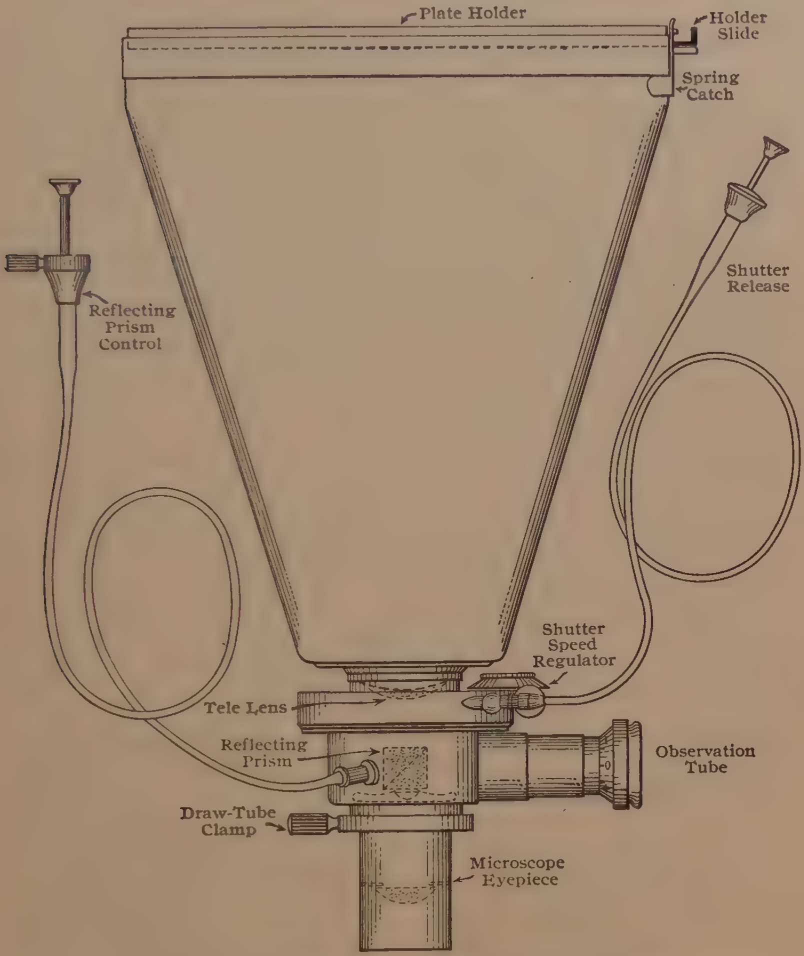
Plate Holder
Holder Slide
Spring Catch
Shutter Release
Reflecting Prism Control
Shutter Speed Regulator
Tele Lens
Reflecting Prism
Observation Tube
Draw-Tube Clamp
Microscope Eyepiece

FIG. 105. "Makam" Photographic Eyepiece (Leitz).

has a fixed projection distance of 250 mm. It uses $3\frac{1}{4} \times 4\frac{1}{4}$-inch plates, but is supported on an upright arm rising from a base plate, on which the microscope must be placed whenever a photograph is required.

Such "photographic eyepieces" are of particular value in chemical work, since they permit instantaneous photographs of moving or changing objects. The plate may be made ready in the camera, and a suitable field may be located and focused by means of the observation tube. If the specimen is undergoing some alteration, it may be watched carefully and kept in focus until it presents the desired appearance, when the exposure is "snapped." If necessary, the reflector of the observation tube may be left in place during a time exposure, so that the focus may be maintained sharp and may be terminated if any marked change takes place in the specimen. With a powerful light source, exposures of less than $\frac{1}{25}$ of a second are feasible; and by having a number of plate holders ready, these may be made in succession, at intervals of a few seconds.

Because of the ease with which they may be attached to any microscope, photographic eyepieces are very convenient for recording appearances encountered in visual examinations. The camera is brought to the microscope, and there is no risk of losing the particular field under observation. The greatest advantage of these instruments lies in their use for photography of changing objects, as in the study of solution, fusion, crystallization from liquids or solids, decomposition by heat or chemical means, flocculation of colloids, and similar phenomena.[9]

Cameras with adjustable bellows are particularly suited to work where magnification must be readily adjustable, and where critical focusing is necessary. Since the microscope will be used at least part of the time in the erect position, a vertical camera is

[9] It is possible to use any small hand camera for photomicrography, by focusing it for "infinity" and mounting it over the ordinary eyepiece, the microscope having previously been focused in the usual manner. The camera functions as an eye accommodated for infinity, as is normally the case in microscopic vision. The film is analogous to the retina, and a real image is formed upon it by the eye lens of the eyepiece and the camera lens, acting jointly. (*cf.* Fig. 1.) A light-tight adapter is necessary, together with a support for the camera. These accessories may be obtained for the "Leica" camera from Leitz, or the Anso "Memo" camera from Bausch & Lomb. Both these small cameras use standard motion picture film, and a number of pictures may be taken in rapid succession.

desirable. A number of different models are available, of which that shown in Fig. 106 is typical.

The vertical support rod should preferably be rotatable, to swing the camera to one side for direct visual observation, and graduated to permit duplication of adjustments of the bellows extension. The camera proper must be connected with the microscope by a light-tight double sleeve. The illuminating apparatus with auxiliary condenser and field diaphragm may be arranged on the table beside the camera support, or the whole system may be aligned upon an optical bench. The outfit should be placed on a very low table so that the user may conveniently look down upon the camera when focusing.

Rigid construction is necessary, since the camera on its long support rod tends to act as an inverted pendulum, and is more susceptible to vibration than if it were in a horizontal position. For this reason, a number of firms manufacture cameras which may be used in either the vertical or the horizontal position; guy rods are used to give sufficient steadiness in the upright position. For extremely high magnifications, with long bellows extensions, the horizontal position of the camera is preferable, both because of the greater steadiness of the apparatus and the greater ease of observing the focusing screen. In general, such high magnifications are not necessary in the case of most fluid specimens, and by proper mounting of the object the microscope may be used horizontally. If a vertical position of the microscope is considered essential, a 45° prism may be placed at the eyepoint, and connected to a horizontal camera.

The camera back, which carries the focusing screen, should be constructed so that the plate holder may be put in place without manipulation of inconvenient fasteners and without jarring of the apparatus. The slide of the plate holder, which is withdrawn to uncover the plate, should work freely, without sticking, for the same reason. If adapters or " kits " are used to accommodate small-sized plates, they should be well constructed so as not to fall out of place or warp.[10]

Vibration is a serious hindrance to photomicrography at high magnifications, especially if the laboratory is in the same building

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[10] For most work a camera which accommodates 5 × 7-inch plates is adequate; it should be equipped with kits for 4 × 5 and $3\frac{1}{4} \times 4\frac{1}{4}$-inch plates, since these sizes are standard and easily obtainable.

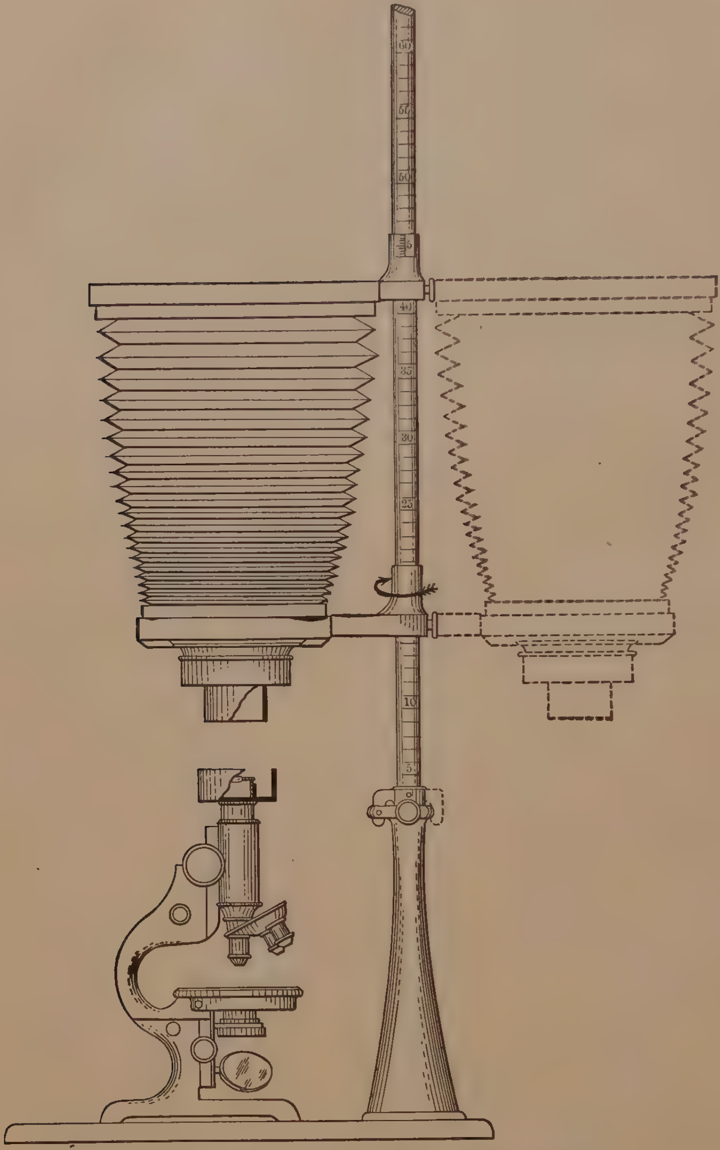
40
35
30
25
10
5

Fig. 106. Vertical Camera for Photomicrography.

with machines or blowers or is near heavy traffic. Numerous schemes have been proposed to insulate the apparatus from external vibrations.[11] Tables with tops resting on springs or suspended by springs from the ceiling are very effective and are reasonably convenient to use as supports for all types of apparatus. Tennis balls or inflated tire inner tubes have been employed with success to cushion the false top of a large table. Pads of heavy "deadening felt" at least an inch thick, placed under each table leg and under each support of the optical bench, absorb extraneous vibrations fairly satisfactorily.

Some of the injurious effect of vibration may be mitigated by taking pains to have the specimen rest firmly on the stage, held by clamps if necessary, and to avoid an excess of mounting liquid of low viscosity. If an aqueous preparation is being studied, the water by which particles are surrounded, and on which the cover-glass floats, may vibrate more freely than any other part of the system and cause marked blurring of the image. Excessive magnifications and long bellows extensions are to be avoided if vibration is present. Any "creep" in the fine adjustment, after focusing has been completed, should be taken up carefully by focusing upward slightly. The apparatus should be protected from stray heat from the arc, or the focus may shift as the exposure continues and the stand of the microscope expands unsymmetrically.

The choice of a field to be photographed has already been emphasized as requiring experience and judgment on the part of the microscopist. Objects which appear the same in all parts are rare indeed, and the field which is selected as representative must either be large enough to include all significant variations of appearance in their true relative frequency, or it must be restricted to some one important feature which is to be emphasized. In the latter case, it is frequently desirable to show, by a photograph at lower magnification, the relationship of the detail to the object as a whole.

As a result of preliminary visual examination the microscopist should have clearly in mind the characteristics of the object which it is particularly important to record clearly and exactly. In the light of this study he should be able to secure the illumination which will render these features most vivid, and to choose a field which shows them in unmistakable and typical form. This may require more time than all the rest of the photomicrographic operation, but it is essential if the photograph is to be of real use as a record and a standard for future reference.[12]

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[11] Spring suspension is used on the optical bench of the large Leitz and Bausch & Lomb cameras, and on the metallographs of these firms.

Benedicks: *Metallographic Researches* (1926), p. 152 discusses the elimination of vibration by spring suspension of the optical bench of the apparatus.

The choice of lenses and magnification depends largely upon whether a general view or a particular detail of structure is to be recorded. The area included in the field is governed mainly by the magnifying power of the objective, and if a large area is to be photographed an objective of long focal length must be chosen. This field is imaged on the photographic plate over an area which depends on the magnification of the eyepiece and the length of the camera. If the picture is to cover a large plate, a high power eyepiece and a long bellows will need to be used. Since the edges of the image are likely to show aberrations, they are sometimes cut off by image diaphragms placed just in front of the plate holder. These diaphragms[13] consist of metal plates with square or round openings of various sizes, and give a sharp boundary to the image. It is also very useful to have marked on the focusing screen the areas of plates of various dimensions, so that there can be no doubt as to whether the object is truly centered and can be included by a plate of the dimensions used.

The considerations governing the limits of magnification, resolving power versus depth of focus, and flatness of field, have already been discussed (Chapter I), as have the relative merits of different types of objectives and eyepieces. Whether a given magnification should be obtained by a low-power eyepiece and a long projection distance or a high-power eyepiece and short bellows is largely a matter of trial with the particular lenses at hand. Certainly the magnifying power (and numerical aperture) of the objective should be as high as is consistent with the depth and breadth of the field to be shown; an objective with an aperture diaphragm is a great convenience for this reason.

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[12] One of the most valuable methods of developing a critical sense in photomicrography is to study the best work on various types of objects. The photomicrographs shown in the following publications indicate what to strive for:

Paper Fibers — Herzberg: *Papierprüfung* (Berlin, 1927); Sutermeister: *Chemistry of Pulp and Paper Making* (John Wiley & Sons, New York, 1919).

Textiles and Paper — Herzog: *Mikoskopischer Atlas der technisch wichtigsten Faserstoffe.* I Tl. (München, 1908.) (Some of the illustrations of this work are reproduced in Mathews' *Textile Fibers* (1924).

Leather — Wilson: *Chemistry of Leather Manufacture* (1928).

Metals — Sauveur: *Metallography and Heat Treatment of Iron and Steel* (1926).

Pigments — Green: *Jour. Franklin Inst.* **192**, 637 (1921).

[13] Obtainable from Zeiss.

The **magnification** is determined roughly by the formula given on page 244, which serves as a guide in the choice of lenses. The exact magnification should be obtained experimentally, and should be adjusted to some round figure. The standard magnifications recommended by the American Society for Testing Materials (**E 2–24**) should be adhered to as far as possible. These are as follows: 10, 25, 50, 75, 100, 200, 500, 1000×. The exact magnification and dimensions of the real field can be determined by replacing the specimen by a stage micrometer, the image of which is projected on the focusing screen. A scale is superposed on this image, and the size of the micrometer divisions is measured carefully. (See page 406.) By varying the extension of the bellows the magnification may be adjusted very exactly over a considerable range, and may be duplicated if the variables which control it are recorded. By marking on a strip of paper the size of each division of the stage micrometer, a scale may be prepared which can be transferred to each print if desired.

Focusing is really a matter of choice, and depends to a large extent upon the judgment of the observer. Of course, serious deviations from a sharp focus are easily detected, but within a considerable range it is frequently difficult to decide what is the correct position of focus. The entire aspect of the object may be altered, without much noticeable change in sharpness, by a slight movement of the fine adjustment. White may be made to appear black, and structures near the limit of resolution may have their apparent dimensions increased or decreased, by focusing alone. If oblique illumination is used, shadows may appear as realities. With dark field illumination diffraction patterns may obscure true structure or give spurious images.

If the object possesses appreciable thickness, the microscopist is further confronted with the problem of selecting which particular plane he will bring into focus, and which features he will leave blurred and indistinct. In the photomicrography of powdered materials which contain grains of widely different sizes, the apparent particle size of the material may be varied over wide limits by focusing either on the large particles or on the small ones which are in a lower plane. For the above reasons the microscopist should familiarize himself with the structure of the specimen by thorough visual study, in order that he may choose a position of focus which truly depicts its details. Although it is often suggested that several exposures, each at a different focus, be combined on the same negative, the results of such procedure are not very satisfactory.[14]

The operation of focusing may be carried out by means of a ground-glass screen in the plane where the photographic plate will later be placed. Slight discrepancies between the positions of the ground surface of the focusing screen and the photographic emulsion are not serious, since the image-forming rays have great depth of focus. However, care should be taken not to place the holder of the ground-glass wrong side out, for this may result in a displacement of several millimeters.

In photomicrography at low or medium powers, the point of best focus can readily be located by careful and critical study of the image on the ground-glass of the camera. If curvature of field is evident, the most important features of the object should be moved to the center of the field, and focused as sharply as possible. The outer zones will at least serve as a background, and may be trimmed off the print if they are too badly blurred.

The " grain " of the ground-glass screen may obscure the finest details, and the intensity of illumination may be very low at high magnifications, hence clear-glass screens are commonly used for the most accurate focusing. The real image from the microscope cannot be observed in a transparent surface, so a **focusing glass** is necessary. This consists of a well corrected simple magnifier in a mounting of variable height. It is adjusted so that, when the mounting is placed in contact with the outer surface of the focusing screen, the inner surface will be exactly in focus, as will also the image in this plane.[15] Very faint images, such as are obtained at extreme magnifications, may be made clearly visible by the use of a focusing glass, and the effect of the slightest movement of the fine adjustment is easily noticeable.

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[14] This method is described by Petersen: *Zeits. wiss. Mikros.* **41**, 365 (1925).

[15] Such a focusing glass might be considered as analogous to a positive eyepiece.

On account of the " lag " in the movement of some fine adjustments it is well to focus upward for the final setting, and to check the focus after some minutes, before inserting the plate and making the exposure.

Photographic Operations. — The microscopist is at a distinct advantage if he has had some experience with photography (other than " pushing the button ") before he undertakes photomicrography. Otherwise, not only has he to transfer his knowledge of microscopy to photographic apparatus, but he must also acquire a fairly detailed understanding of the art and science of photography under somewhat difficult conditions.[16]

Most work such as the chemist and technical microscopist encounter does not have to do with colored objects, and requires strong contrasts rather than delicate rendition of faint shadows. Transparent crystals, colorless pigments or fillers, undyed textile or paper fibers, and most metals present little more than a black-and-white image, frequently somewhat faintly outlined. Plates, developer, and printing paper should be chosen with this in mind, except in special cases.

For general photomicrography of uncolored objects, fine-grained, slow, " contrasty " plates or cut films are to be preferred. " Process " or " commercial " emulsions are particularly useful because they give fine dense blacks, and prevent graying of light areas in the print. If a green color filter, as used with achromatic lenses, is employed, the sensitivity of plates of this type will be found rather low, and an " orthochromatic " emulsion is desirable. This will give reasonably correct rendition of blues, greens, and light yellows, but orange and red will photograph too dark. If the specimen is colored in these latter hues, " panchromatic " plates or films may be necessary; they may be obtained in " process " or " commercial " grades for high contrast. A yellow filter may be used to prevent undue brightness of blues, though this is not so necessary if the light source is an incandescent lamp instead of an arc.[17]

Contrast may be further aided by the use of a suitable developer, such as one containing metol and hydroquinone. If photomicrographs are made only occasionally, developers in tablets or tubes are most convenient, but if much work is done it is better to have on hand a concentrated stock solution developer which can be diluted for use.[18] The advantages of a developer which works equally well for plates, films, or papers are obvious.

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[16] As a simple and concise introduction to the subject, " *The Fundamentals of Photography* " by C. E. K. Mees (published by the Eastman Kodak Co.) is highly recommended.

[17] The use of color filters for contrast or transparency has been discussed on page 101.

The **time of exposure** is an important factor in controlling the density and contrast of the negative. In general it is better to over-expose slightly rather than to under-expose the negative, since the darker parts of the image may not otherwise be recorded. The determination of the correct exposure time should be based on preliminary trial exposures, unless the conditions are such that previous experience may be utilized as a guide. Several methods are available:

1. The slide of the plate holder may be drawn out completely, so as to uncover the entire plate. An exposure of, say, 1 second is made; the slide is pushed in about 1 cm. and a second exposure of 1 second is made. Another centimeter strip of the plate is covered, and an exposure of 2 seconds is made, and so on, doubling the exposure each time. When the negative is developed for the normal time at the temperature specified, the strips corresponding to the several exposures will be of different densities and will show varying contrast. From them the best time can be selected, and used for the final exposure.

2. Instead of making a series of separate exposures of progressively increasing length, a single exposure may be made, with illumination of graduated intensity. To do this a neutral-tint filter of graduated transparency[19] is placed in front of the plate, and an exposure which is thought to be much too long is given. Each of the areas screened by the different " steps " of the graduated filter receives an illumination equivalent to the fractional part of the exposure which is marked on that " step." From an examination of the normally developed negative the ideal exposure time can be selected.

3. If a fairly close guess can be made as to the proper time of exposure, by judging the brilliancy of the illumination on the ground-glass, a trial exposure of the entire plate may be made. This is developed at the normal temperature, and the time of first appearance of the image is noted. This

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[18] The authors have found R. L. Boyd's formula (Hind and Randles: *Handbook of Photomicrography*, p. 273) very satisfactory for all purposes, and of excellent keeping qualities.

Metol	14 grams
Hydroquinone	56 grams
Potassium bromide	12 grams
Warm water	1000 cc.

When dissolved, add sodium sulphite (crystals crushed small), 400 grams. To the white pasty mass add caustic soda, 40 grams.

For plates or papers: take one part of the stock solution and add 7 parts of water. Make fresh each time used.

Watkins factor: 16. Normal development time: 3 min. at 60° F.

[19] Such as the " Goldberg Wedge," of Zeiss; or Mayer's " Dremmeter," obtainable from Chas. G. Willoughby, Inc., 110 W. 32 St., New York.

should be roughly equivalent to the normal development time divided by the " Watkins factor " of the developer.

4. Further indications of incorrect exposures are: first image flashing up in brownish tones, over-exposed; faint image even if development is pushed until general grayness results, under-exposed.

Once the correct exposure time has been determined for a given set of conditions, all the data may be recorded and used as a guide for further work. Such information can be applied to photomicrographs taken under other conditions, either by the use of rather elaborate formulas or by means of factors which depend on the different variables.[20] The normal time of exposure varies as follows:

Inversely as the square of the numerical aperture of the objective and of the condenser.
Directly as the square of the magnification.
Directly as the filter factor.
Inversely as the speed of the plate.
Inversely as the actinic intensity of the light source.

It is also affected by the light-transmitting powers of the various lenses of the microscope and illuminating system, and by the color and transparency of the object. Fortunately, the latitude of exposure permissible with modern plates and developers is such that reasonably good results can be secured with exposures which are only approximately correct. Of course, for routine work under constant conditions a high degree of standardization is possible.

Development should be carried out by time or by the " factorial " system, with due regard to the character of the negative which is desired. In general, slight over-development is preferable to under-development, in order that all the latent image may be utilized. If the factorial system is used, the time from the immersion of the negative in the developer to the first appearance of the image is noted; this, multiplied by the " Watkins factor " of the developer, gives the time required for full development, irrespective of temperature and over- or under-exposure within moderate limits.

If the negative has been over-exposed, it should nevertheless be developed fully, unless it is possible to make a new exposure. The developed negative may be reduced in density, with some increase in contrast, by means of Farmer's reducer.[21] Under-exposed negatives are of little value, and should be re-taken if possible.

Fixation is carried out according to the usual procedures; a chrome alum bath is to be preferred on account of its pronounced hardening effect on the emulsion in warm weather.[22] Thorough washing is necessary after fixing the negatives; 30 minutes in running water is sufficient.

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[20] Petrunkevitch: *Anat. Record* **19**, October (1920); *Amer. Naturalist* **55**, (1921).

[21] A 20 per cent solution of sodium thiosulphate (hypo), in which enough potassium ferricyanide has been dissolved to give a lemon yellow color. Use on fixed and washed negatives. Wash the negative thoroughly after reduction.

Printing of positives may be carried out so as to accentuate or decrease the contrast of the negative. Glossy paper should be used; the grade " for normal negatives " is generally satisfactory, but for very thin, " flat " negatives a more contrasty paper may be necessary, whereas for bringing out structure in shadows a paper with less contrast should be selected. The detail in the prints will be much clearer if they are dried on " ferrotype plates."

The developer described above is very satisfactory for papers; trial exposures are hardly necessary in most cases, but may be made on strips of paper if required. The development of the positive image is followed visually, and is terminated when the desired density is reached. A brownish image which flashes up in a few seconds indicates too long exposure; normal exposure will give deep blue-black tones. Fixing is done in a bath of the formula given above; 15 minutes is sufficient. This is followed by washing in running water for half an hour.

Although in special instances it may be necessary to deviate from the photographic materials and methods outlined above, a great deal may be said in favor of the practice of standardizing the procedure on the basis of as few types of plates, papers, and solutions as possible, and of striving to obtain good results by correct exposure and development rather than by " doctoring " poor negatives.

SPECIAL METHODS OF PHOTOMICROGRAPHY

Photomicrography in colors, by means of " Autochrome " or similar plates, is even simpler than in macroscopic photography, since the most important factors, illumination and exposure time, are thoroughly under control. Once the correct exposure has been determined, excellent results can be secured in future work. The colors are never as brilliant nor as " saturated " as those of the original, and the finished transparencies are rather opaque for projection as lantern slides, but striking and accurate renditions of polarization colors, staining reactions, and similar phenomena are possible. The procedure

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[22] The authors have found the following formula very satisfactory for both plates and papers:

A —	sodium thiosulphate (hypo)	1000 grams
	sodium sulphite, anhydrous	65 grams
	water	3 liters
B —	chrome alum	130 grams
	glacial acetic acid	24 cc.
	water	2 liters.

Mix 1 part of B with 4 parts of A. The solutions keep well when mixed, but much longer separately. A fresh bath should be used if fixation is slow, or if sediment has accumulated. Fix for about twice the time required to clear the plate, or about 15 min.

is not complicated, and very specific directions are supplied by the manufacturers of the plates.[23]

Photomicrography with crossed nicol prisms, of various polarization phenomena, is often very useful in the study of anisotropic materials such as crystals, textile and paper fibers, plastics, and plant or animal tissues. The analyzer nicol prism may be placed above the eyepiece, or in the body-tube; if in the latter position it should be equipped with a correction lens of the best quality, to eliminate the aberrations caused by the introduction of the nicol prism in the path of the image-forming rays. Particular attention should be paid to focusing, since otherwise haloes and diffraction patterns may be objectionably prominent in the image. The various hues of polarization colors may be rendered fairly accurately by orthochromatic plates, but panchromatic emulsions are better. Very beautiful results may be obtained with "autochrome" plates.[24]

Photomicrography of opaque objects by vertical illumination is greatly simplified by the convenient design of the larger metallographic microscopes, which are constructed primarily as photographic outfits (page 129). The principal problem is that of illumination; resolution without loss of contrast is essential, and glare must be minimized as much as possible.

Photomicrography with monochromatic ultraviolet light represents the utmost in microscopic resolution, but presents many manipulative difficulties. The light source must be strictly monochromatic, of wavelength 275 mμ, and of high intensity. All lenses must be of quartz. Focusing is of necessity indirect; fluorescent screens can be used in conjunction with special eyepieces, but the image is very faint. The specimen can be focused visually by monochromatic green light, and a certain previously determined shift of the fine adjustment made to render it in focus for ultraviolet light. In any case, successive exposures, each at slightly different focus, are desirable, in order that the most representative negative may be selected. In fact the microscopist is literally working "in the dark," and can judge his methods only by the final product.[25]

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[23] Lumière Autochrome Plates: R. J. Fitzsimmons Corp., 75 Fifth Ave., N. Y.
Agfa Color Plates: Agfa Products, Inc., 114–116 E. 13th St., New York.

An excellent discussion of the technique of photomicrography in natural colors is given by Curtis: *Jour. Amer. Ceram. Soc.* **11**, 609–32 (1928).

[24] Northall and Laurie: *Photog. Jour.* **63**, 48 (1923).
Curtis: *loc. cit.*

[25] For a discussion of the apparatus and technique of ultraviolet photomicrography, the reader is referred to the following publications in addition to those already listed:

Köhler: Mikrophotographische Untersuchungen im ultravioletten Licht. *Zeits. wiss. Mikros.* **21**, 129 (1904).

Barnard: Microscopy with Ultraviolet Light. Glazebrook's *Dictionary of Applied Physics.*, Vol. IV, pp. 237–45.

Jour. Roy. Micros. Soc., **287**, 91–101 (1929).

Lucas: An Introduction to Ultraviolet Metallography. *Pamphlet No.* 1576–*E*, issued with *Mining and Metallurgy*, June, **1926.**

Martin and Johnson: *Jour. Sci. Instruments* **5**, 337, 380 (1928).

Photomicrographic motion pictures may serve as a convenient substitute for microprojection or personal experimentation, but their chief scientific justification is as a means of recording phenomena which are not adequately visible to the eye.[26] Successive exposures, at relatively long intervals, of an imperceptibly slow process may be projected at normal speed to summarize in a few minutes the changes of hours or days and to give a unified concept of their progress.[27] Ultra-rapid exposures may be made of appearances which are altered too rapidly to be followed by the eye or to be " snapped " by a single instantaneous exposure at exactly the proper stage; these may be projected in the form of " slow-motion " pictures, so that a detailed analysis of the entire sequence is possible.[28] Statistical studies of complicated changes in form[29] or number may be made on the successive exposures of motion picture film.

In any of the above applications it is of course essential that the field and the focus should be under control during the exposures. Vibration from the camera mechanism is seriously objectionable, particularly at high magnifications, and a separate support may be necessary. Although there is no doubt that a large apparatus using standard film (such as that of Leitz) is desirable for critical work, simpler outfits may be improvised, using a small hand camera.[30]

A viewing eyepiece, such as the " Mikrophot " of Zeiss, which sends about one per cent of the light to the eye and the remainder to the film, may be arranged so that observation may be continuous, even while the camera is running. Another scheme is to attach the camera to one tube of a binocular eyepiece, the other tube being used for finding and focusing the object. Careful preliminary tests for coincidence of visual and photographic fields and foci are essential in any case.

APPARATUS AND METHODS OF MICROPROJECTION

The simplest apparatus for microprojection consists of an ordinary microscope, a lamp with auxiliary condenser, and a screen. For chemical work it is generally necessary that the microscope screen be used in the erect position, so a reflector is required above it.

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[26] Deischa: *Zeits. Kryst.* **50**, 24 (1911).

[27] Kraemer: *Second Colloid Symposium Monograph* (1924), p. 57.
Weiser and Cunningham: *Sixth Colloid Symposium Monograph* (1928), p. 330.
Storch: *Zeits. wiss. Micros.*, **46**, 21–44 (1929).

[28] Tuttle: *Trans. Amer. Soc. Motion Picture Eng.* **1927**, 213.

[29] Wightman and Trivelli: *Ind. Eng. Chem.* **17**, 164 (1925).
Ecket and France: *Jour. Amer. Ceram. Soc.* **10**, 579 (1927).

[30] Köhler: Mikrokinematographie und biologische Filmaufnahmen. In Abderhalden's *Handbuch der biologischen Arbeitsmethoden.* Abt. II, Teil 2, Heft. 3, pp. 1109–84.
France: *Jour. Amer. Ceram. Soc.* **9**, 67 (1926).
Metzner: *Das Mikroskop* (1928), p. 488.
Scheminzky and Kann: *Zeits. wiss. Mikros.* **45**, 11–33 (1928).

The "Euscope" of Bausch & Lomb is such an outfit; it is designed for use by a single observer, who looks directly at the projection screen, instead of into the microscope. The screen is contained in a viewing box, so that it is shielded from external light and a darkened room is not required. The image is formed at a fixed distance (about 250 mm. from the eyepiece), and is of apparently the same size as in the microscope. By using a translucent screen, the image can be viewed from both sides; it is too small to be studied by more than a very small group. A photographic plate holder may be put in place of the viewing screen, so the instrument can be used as a camera.

A number of such individual projection devices are on the market, most of them designed to project a real image on a sheet of paper, to be traced for drawing.[31] Some necessitate a horizontal position of the microscope, and all require some sort of hood to keep light from the projection surface. They may be improvised by means of reflectors appropriately placed, but are not particularly useful in chemical work.

Projection of microscopic objects to render them visible to a large group of observers requires more elaborate apparatus, particularly as regards the illuminating system. For class room use, however, where instruction in the manipulation of the microscope is as important as observation of the final image, it is highly desirable that the projection outfit should not be so complex as to mystify the student or conceal the methods of illumination which are used to bring out certain features of the object. For this reason the ideal outfit for microprojection should utilize an ordinary microscope with standard substage condenser, polarizer, vertical illuminator, and other accessories.

Either of the outfits by Leitz is very satisfactory in this respect. The earlier model utilizes an automatic arc lamp with auxiliary condenser of great light-grasping power and variable focal length. A field diaphragm is placed in front of this condenser, and serves to cut off useless light, and to protect from heat the portion of the specimen which is outside the field. The light source is imaged in the opening of the substage condenser, according to Köhler's method of illumination. An adjustable base plate, mounted on the same optical bench with the illuminating system, permits the microscope to be raised or lowered for alignment in a vertical or a horizontal position. A somewhat similar outfit may be obtained from Bausch & Lomb or Zeiss.

The latest projection apparatus Xa of Leitz has proved to be of almost universal application in demonstration of the phenomena of chemical microscopy (Fig. 107), particularly since any type of monocular microscope may be used in connection with it. It consists of a cast-iron base, on which the microscope is placed, and an adjustable light source. The automatic arc lamp *R*, in a light-tight housing, may be centered by means of the screws *SS'*, and may be placed at any height on the vertical support rods, so as to be aligned with a vertical illuminator or with the axis of a microscope in the horizontal position. A hard-glass condenser *C* with iris diaphragm functions as a secondary light source of variable size, and images the crater of the arc in the aperture of the illuminating system of the microscope according to Köhler's method.

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[31] These instruments function entirely differently from the "drawing camera" (page 391), which does not project an image.

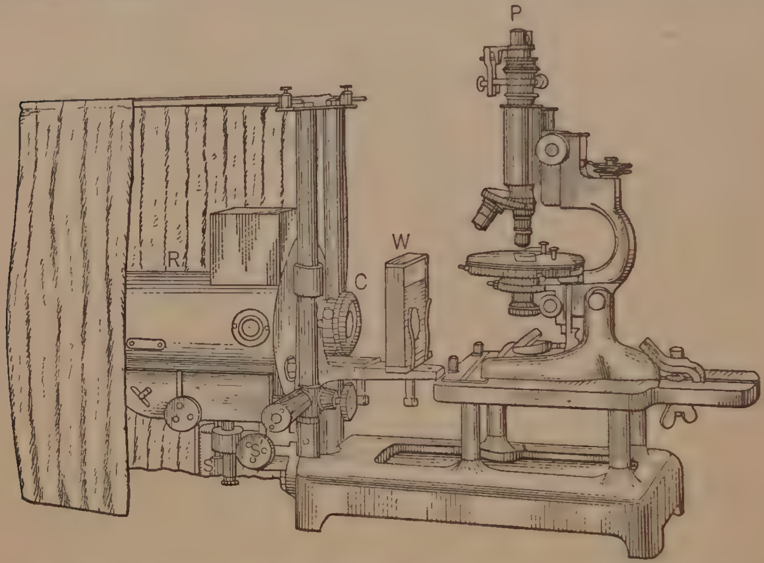

FIG. 107. Microprojection Apparatus (Leitz) with Chemical Microscope (Bausch & Lomb).

A 45° totally reflecting prism *P* in a hinged mount is placed above the eyepiece, to reflect the image-forming rays to the screen.[32] Heat-absorbing glass (page 108) in a small water cell *W* is essential if delicate objects are being projected, or if a polarizing nicol prism is used.

The apparatus is shown as employed for projection with the ordinary chemical microscope in an upright position, using crossed nicol prisms. The entire outfit is exceedingly flexible, and permits rapid transition from one type of illumination to another, with as great manipulative convenience as in ordinary visual microscopy. With an Abbe condenser or a prism-type

[32] The prism made by Zeiss (for photomicrography with an upright microscope and a horizontal camera) is preferable to that of Leitz. It is attached to the upper end of the draw-tube, instead of to the eyepiece, and is readily adjustable in height or to give space for a cap nicol prism.

vertical illuminator, four- or five-foot images are readily obtained; with polarized light they must be smaller on account of the decreased brightness. Interference figures or Abbe's diffraction phenomena may be projected by means of a Bertrand lens (page 289) or a low-power objective in the lower end of the draw-tube.

The brilliancy of the image is the most important limitation in microprojection. The light should be concentrated so as barely to fill the aperture of the substage condenser. A translucent screen, of thin white tracing cloth, between the instrument and the observers is most satisfactory, especially if the room is incompletely darkened.

CHAPTER IX

THE STUDY OF DOUBLY REFRACTIVE MATERIALS BY MEANS OF THE POLARIZING MICROSCOPE

A compound microscope equipped with polarizing apparatus has its usefulness extended far beyond that of mere magnification. In addition to its revelation of fine structure, it becomes an instrument for the observation of properties and the determination of constants which are of the utmost significance in chemical investigations and are of wide application in other fields. The polarizing microscope is the instrument *par excellence* for such studies; it takes the place of other less universal apparatus, utilizes easily prepared microscopic specimens, and permits easy correlation of form and optical properties.

In practically all investigations of crystalline or pseudo-crystalline materials the polarizing microscope is of potential value, and may yield information unobtainable by other means, since it deals with *inherent* properties as well as with external appearances. Examinations with polarized light do not involve any alteration of the specimen, yet may reveal structural features better than elaborate chemical treatment or staining methods. The specific applications cited on pages 305, 328 are suggestive of the many possible uses to which the instrument may be put, but it should be realized that there are almost no microscopic studies in which polarized light is not worthy of trial.

The extent and accuracy of the observations made with a polarizing microscope depend upon three factors: the knowledge and skill of the observer, the perfection of the apparatus, and the character of the specimen. In no other field of microscopical technique is a thorough comprehension of fundamental optical principles so essential, and " rule of thumb " procedure so likely to lead to error. Simple qualitative observations, intelligently made, are likely to be more useful than elaborate determinations based on half-comprehended directions. Before undertaking such studies, the reader is urged to refresh his knowledge of the properties of polarized light, and to study several of the standard works on the polarizing microscope.[1] Practice on the various experiments outlined will give first-hand knowledge of the phenomena which they are designed to illustrate, and experience in the necessary manipulations of the microscope required for their exhibition.[2]

THE POLARIZING MICROSCOPE

In addition to the usual lens system of objective and eyepiece, polarizing microscopes possess a number of other optical features. It is rarely practicable to convert a " biological " microscope by attaching polarizing apparatus to it; the resulting instrument will only serve for the very simplest observations and will be inadequate for use in most chemical work.

The items of equipment which are more or less essential to a polarizing microscope are listed below, in the order of their importance:

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[1] Winchell: *Elements of Optical Mineralogy*, Part I (John Wiley & Sons, New York, 1928).

Ford: *Dana's Textbook of Mineralogy*, Part IV, Section III (John Wiley & Sons, New York, 1922).

Kraus and Hunt: *Mineralogy* (McGraw-Hill Book Co., New York, 1928), Chap. XI.

Groth-Jackson: *Optical Properties of Crystals* (1910).

Johannsen: *Manual of Petrographic Methods* (McGraw-Hill Book Co., New York, 1918).

Wright: *The Methods of Petrographic Microscopic Research.* Carnegie Institution Publication No. **158** (1911).

Tutton: *Crystallography and Practical Crystal Measurement*, 2 Vols. (MacMillan & Co., London, 1922).

Köhler: *Die Verwendung des Polarizationsmikroskops für biologische Untersuchungen.* Abderhalden's *Handbuch der biologischen Arbeitsmethoden* (Urban & Schwarzenberg, Berlin, 1928), Abt. II, Tl. 2, Heft. 2, Lief. **191.**

Ambronn and Frey: *Das Polarisationsmikroskop* (Akademische Verlag, Leipzig, 1926), Band **5**, *Kolloidforschungen in Einzeldarstellungen.* Herausgeben von R. Zsigmondy.

Rinne: *Kristallographisches Formenlehre* (M. Janecke Verlag, Leipzig, 1922).

Groth: *Elemente der physikalischen und chemischen Krystallographie* (R. Oldenbourg, Munich, 1921).

Leiss and Schneiderhöhn: *Apparate und Arbeitsmethoden zur mikroskopischen Untersuchung kristallisierter Körper* (Franckh'sche Verlag, Stuttgart, 1914), Teil X, *Handbuch der mikroskopischen Technik.*

[2] In addition to the examples given, various other experiments will suggest themselves to the reader of books such as Groth's and Köhler's works. The exercises given by Winchell are particularly valuable; the necessary mineral specimens, in the form of thin sections, may be obtained from W. Harold Tomlinson, Petrographic Laboratory, Swarthmore, Pa., or from E. Leitz, Inc.

1 — Polarizer and analyzer.
2 — Mountings to permit rotation and definite orientation of polarizer and analyzer.
3 — Crosshaired eyepiece, fixed so as to indicate orientation of polarizer and analyzer.
4 — Rotating graduated stage, with provision for adjusting rotation concentric with the center of the field.
5 — "Compensators," with provision for inserting them in a slot or otherwise.
6 — Condenser above the polarizer.
7 — Bertrand lens.

The greater the number of these features incorporated in the microscope, the greater will be the variety of qualitative and quantitative determinations which can be made with it. The first five are practically indispensable. If these are not provided, most observations are extremely inconvenient, or even impossible. Proper design of the stand, to facilitate accurate manipulation of the essential equipment, is a requirement of hardly less importance.[3]

The more elaborate polarizing microscopes are often called petrographic microscopes, and the simpler types, chemical microscopes, but the former have no optical features which are not of as much use in chemical as in mineralogical studies. However, the stands of certain types of petrographic microscopes are not convenient or suitable for chemical work, or general crystallographic studies.[4]

Polarizer. — For illuminating the specimen with polarized light, some form of polarizing apparatus is necessary. A **nicol prism** mounted in the substage ring is most commonly employed. Although the external shapes of such prisms vary considerably, their action depends primarily upon the strong double refraction of the calcite from which they are constructed. A ray of ordinary unpolarized light may be considered to possess wave motions in all possible planes parallel to the direction in which it travels.

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[3] Wright: *op. cit.* pp. 10–13, discusses the requirements for a polarizing microscope.

[4] The stands and equipment of several types of polarizing microscopes particularly suitable for use in the chemical laboratory are described in Chapter II.

On entrance into a doubly refractive material, of which calcite is a very striking example, the lateral vibrations are resolved into components in two perpendicular planes only. Each of these components consists of polarized light, with all its vibrations in a single plane. If the angle of incidence is properly chosen, the component rays travel along different paths, and may be separated for use as polarized light. A nicol prism consists of a crystal of calcite, of the requisite shape, which has been cut in two diagonally and recemented with Canada balsam. One of the polarized rays strikes the cementing material so obliquely that it is totally reflected and passes to the side of the nicol prism where it is absorbed at the blackened surface. The other polarized ray encounters the layer of balsam at a less acute angle, and passes through it, to emerge at the other end of the nicol prism[5] (Fig. 109).

By passing parallel or slightly oblique[6] rays through such a polarizing prism, light vibrating in a single plane is obtained. On account of the high cost of large nicol prisms, small ones of about one square centimeter cross-section are commonly employed to furnish illumination with plane polarized light. They may be used with either the plane or the concave mirror; the latter is usually preferable, to concentrate the light and to compensate for the necessary loss of over 50 per cent of its initial intensity within the nicol prism. If a condenser is to be used above the polarizer, to supply convergent polarized light, the cross-section of the prism should be as large as the opening of the lower lens of the condenser, in order not to restrict the aperture of the illuminating cone. Ordinarily, small-sized condensers (Fig. 21) are provided for this purpose, instead of large nicol prisms.

The mounting of the polarizer should permit it to be readily removed from the substage, when polarized light is not required, or if other illuminating apparatus is to be used. It is essential that the nicol prism should be replaceable in a fixed position, so that the plane of vibration of the polarized light from it will be definitely oriented with respect to the microscope. This is made possible by a notch and stud in the mountings. Although it is customary to align the polarizer so as to furnish light vibrating "to and from" the observer (parallel to the "6–12 o'clock" crosshair of the eyepiece),[7] its " plane of vibration " should always be ascertained by the microscopist before a new instrument is used. If the nicol prism is rotatable in its mounting, a click should mark the position of permanent alignment.

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[5] Various other types of polarizing prisms are fully described by Johannsen: *op. cit.*, pp. 158–176.

[6] The angular range is about 30°.

Experiment 1. — Hold the polarizer close to the eye and look through the nicol prism at the " high light " on a polished surface (preferably at the reflection from a lustrous black object.) Turn the nicol and note that in certain positions the reflected light is very imperfectly transmitted by it. Light reflected from a surface is partially polarized, and vibrates parallel to the plane of that surface. If the nicol has its "plane of vibration" perpendicular to the reflecting surface, this polarized light will not pass through it. By this means, the " plane of vibration " of any polarizing device may be approximately determined.[8]

Polarization without a Nicol Prism. — In order to illuminate large areas, or to avoid the use of a polarizer in the substage (as in the use of the hot-stage microscope) polarization by reflection may be employed. The simplest arrangement is shown in Fig. 108, by which the light is reflected at the " polarizing angle " from a plate of glass, the under-surface of which is blackened by asphaltum varnish.[9] The intensity of the polarized light is only a few per cent of that of the initial beam, but a powerful light source may be used if necessary.

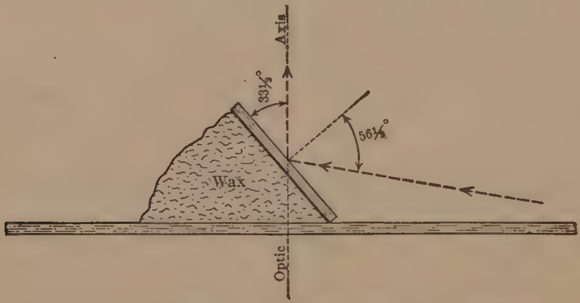

Fig. 108. Obtaining Polarized Light by Reflection.

A number of superposed transparent glass plates may be used as a polarizer; either the reflected or the transmitted light may be employed. Thoroughly clean slides or cover-glasses are satisfactory for this purpose, twelve plates being sufficient.[10]

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[7] The reasons for this practice are discussed by Wright: *Jour. Wash. Acad. Sci.* **5**, 641 (1915).

[8] Distinction must be made between the " plane of *vibration* " and the " plane of *polarization*," which is perpendicular to it. Unfortunately, this is not always clear in published descriptions, which often need to be checked.

[9] Cheshire: *Jour. Queckett. Micros. Club* **8**, 353.

Analyzer. — A specimen illuminated by polarized light appears much the same as if unpolarized light were employed. By placing a second polarizing prism above it, however, any effect which the material may have upon the original plane of vibration of the light will be rendered strikingly evident. This second prism is commonly called the analyzer. A Glan-Thompson prism[11] is usually employed for this purpose. It is mounted so that it may conveniently be set " crossed " with respect to the polarizer, so that their planes of vibration are perpendicular to each other. Under these conditions the analyzer stops all the light that the polarizer transmits.

Experiment 2. — Place the polarizer in position, with its graduation set at zero. Remove the eyepiece and the objective (unless it is a very low power) and adjust the mirror so as to send intense illumination through the polarizer. The direct rays of the sun, an arc, or a concentrated-filament lamp should be employed. Place the analyzer in position, and rotate it, observing that it alternately transmits and stops the light from the polarizer. The positions of brightness and darkness correspond to " parallel " and " crossed nicols," respectively. Note that the light is partially transmitted in intermediate positions, a component of it passing through the analyzer.

Repeat several times the adjustment of the nicols to the crossed position; read the graduated scale, and record the average position of maximum darkness.

The analyzer may be mounted anywhere between the eye and the specimen; above the eyepiece, or in the body-tube just above the objective, are the most common positions. In either case, the nicol is interposed in the path of image forming rays, but its effects on the quality of the image are less pronounced if a " cap nicol " is used above the eyepiece. An analyzer in this position permits a practically perfect image, and is relatively inexpensive. However, it is not satisfactory for use with high-power eyepieces, because it prevents the eye from being placed at the eyepoint, and because of the wide angle of the cone of rays from the eye lens.

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[10] Nelson: *Jour. Queckett. Micros. Club* **14**, 19 (1919).
Johnson: *Jour. Roy. Micros. Soc.* **1921**, 147.
Bryan: *idem.*, **1921**, 149.
A polarizer which functions by reflection from two " piles of plates " is manufactured by Zeiss.

[11] A modified form of the nicol prism, with less oblique end faces.

An analyzer mounted so as to slide in or out of the body-tube of the microscope (*A*, Fig. 23) affords great manipulative convenience, but it alters the focus of the objective, shifts the center of the field, and introduces astigmatism in the image so that horizontal structures in two directions cannot be simultaneously focused with perfect sharpness. These aberrations are more or less corrected by attaching a lens to the end of the analyzer, but are wholly eliminated only by special construction.[12] They are particularly objectionable in photomicrography, but for ordinary work are not serious enough to outweigh the superior ease of manipulation as compared with the cap nicol.

An analyzer which is mounted above the eyepiece is carried on a collar which is attached to the upper end of the draw-tube (Figs. 19, 20, 22). A graduated rim permits its position to be noted. It is essential that the collar should be provided with a notch or stud, so that it may be attached to the draw-tube in a definite position, and it is also necessary that the draw-tube be also fitted with a stud or notch and that it be "keyed" so that it cannot rotate. This construction enables the analyzer to be placed in the proper crossed position relative to the polarizer, without the necessity of readjustment each time it is removed.

An analyzer which slides in the body-tube is usually fixed in its mounting, or at most only rotatable through 90°.

Indication of the Planes of Vibration of Polarizer and Analyzer. — In addition to being adjusted so as to be crossed, the planes of vibration of the two nicol prisms must be known with considerable exactness, and crosshairs are provided in the eyepieces of polarizing microscopes to indicate their positions. The eyepiece is fixed in the proper position by means of a stud which fits in a notch in the draw-tube.

Experiment 3. — Ascertain approximately the planes of vibration of polarizer and analyzer, by the method given above (Experiment 1). Mount the nicol prisms on the microscope; use a low-power objective; illuminate its field strongly, and turn polarizer and analyzer so as to be exactly crossed. Place in the field some well formed crystals of ammonium sulphate (recrystallized according to the procedure given on page 343). Turn the preparation by means of the rotating stage, noting the position of maximum darkness ("extinction") of any given crystal. These positions mark the planes of vibration of the two nicol prisms, and the crosshairs should be exactly parallel and perpendicular to the long edge of the crystal if they are to represent the planes of vibration of the two nicol prisms.[13]

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[12] Wright: *Jour. Wash. Acad. Sci.* **6**, 466–9 (1916).

Rotating Stage. — By means of the circular stage which rotates on an axis coincident with the center of the field, specimens may be orientated in different directions with reference to the planes of vibration of the nicols. The stage should be provided with graduations and a pointer, for angular measurements, and should be large enough to accommodate full sized slides.

Since it is not possible to manufacture objectives and nosepieces which are perfectly and permanently concentric, some provision for adjusting the center of rotation of the stage or the center of the field of the microscope is necessary. Centering screws are provided for this purpose, acting either on the bearings of the stage or the lower end of the body-tube.

Experiment 4. — Fasten a preparation in place on the stage by means of a clip, and examine by transmitted light. Rotate the stage and note that the rotation of the field is excentric, and that the specimen appears to turn about a pivot which is not at the intersection of the crosshairs. Without attempting to bring any particular part of the preparation to the center of the field, adjust the centering screws so as to bring the *center of revolution* nearer to the crosshairs, noting the changes in its position by rotating the stage after each trial. When satisfactory centration has been accomplished, note that any portion of the preparation placed beneath the crosshairs will remain there during a complete revolution of the stage.

Compensators. — (page 283). The most useful compensators are a " 1st order red " plate and a quartz wedge. They are most convenient when mounted in metal, and the direction of vibration of the slower component should be clearly marked on each.

If the compensators are to be inserted in the body-tube, a slot should be provided just above the nosepiece, in a position 45° to the planes of the nicols. Compensators usually do not fit the slot of instruments of different makers; so the dimensions should be known if they are ordered separately.

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[13] If this is not found to be the case, both nicols must be rotated a corresponding amount, or the crosshairs must be realigned with them.

From the approximate observations made in Experiment 1, determine which crosshair indicates the plane of vibration of the polarizer. If the mounting of the analyzer is graduated, note the position of its plane of vibration, in degrees.

For use with a cap analyzer, the compensators may be inserted in a slot in its mounting, just above the eyepiece. If a slot is lacking, a simple compensator such as a " 1st order red " plate in a disk mounting, may be placed between the cap nicol and the eyepiece.

Condenser for Polarized Light. — A condenser mounted above the polarizer is necessary in order to obtain convergent polarized light for the study of interference figures (page 288). It should have a numerical aperture of at least 1.0, and as long focal length as possible (without increasing the diameter of its lower lens beyond the opening of the polarizer) in order that it may illuminate the entire field of a 16-mm. objective. A separable condenser, the top lenses of which are easily swung aside, is desirable in order that larger fields may be illuminated when its lower lens is used alone. On account of its necessarily small field, accurate centration of the condenser is important.

Bertrand Lens. — The observation of interference figures (conoscopic observation), may be carried out by removal of the eyepiece, the back aperture of the objective being viewed directly (Lasaulx' method, page 288). By the insertion of a low-power lens below the eyepiece, a secondary compound microscope is formed (Fig. 111) which is focused on the back aperture of the objective by means of the draw-tube (Bertrand's method, page 289). The Bertrand lens is sometimes provided with a diaphragm, for studies on small crystals.

Objectives for Polarizing Microscopes. — For ordinary work, the usual objectives may be used in connection with a polarizing microscope. It is desirable to have a 4-mm. objective (N.A. > 0.8) available for the examination of interference figures; the other objectives may conveniently have focal lengths of 16 mm. and 32 mm. (or better, 12 mm. and 24 mm.). The numerical apertures and angular apertures of the objectives and the condenser should be known.

The insertion of lenses between the polarizer and analyzer affects the completeness of polarization of the light, and lessens the darkness of the field with crossed nicols. This is due chiefly to the partial depolarization of light by refraction, and is most marked with high-power objectives having many highly curved lens surfaces. As a consequence, a faint uniaxial positive interference figure is observed even when no preparation is under the microscope; its elimination is impossible in objectives and condensers of high aperture.[14]

The polarizing properties of objectives are also sometimes due to the doubly refractive character of the glass or fluorite used in their construction. Ordinarily the effect is very slight; it may be observed by illuminating strongly, crossing the nicols, and rotating the objective. Any alternate brightening and darkening of the field indicates that the objective is unsuitable for accurate investigations with polarized light, though it may be perfectly satisfactory for ordinary observations.

For chemical work the objectives should be mounted on a revolving nosepiece, to permit rapid interchange of high and low powers. They should be accurately parfocal and centered with respect to each other. If these adjustments are carefully made, later centration is rarely necessary.

Testing the Polarizing Microscope. — Before any polarizing microscope is used it should be tested carefully. Testing is particularly important if the nicol prisms are removable from the microscope, as in most chemical microscopes or others to which nicols have been attached. It is well to repeat the tests at intervals of a year or so, since nicol prisms sometimes shift in their mountings.

The rotating stage should be centered, as described in Experiment 4, first with respect to the lowest-power objective of the microscope. The other objectives should then be put in place, and their centrations compared. In general, the centration should be adjusted most accurately for the highest-power objective. If the others are *slightly* off center, no serious hindrance will result in ordinary work.

The centration of the condenser for convergent polarized light should be observed, by noting the position of its iris diaphragm as viewed at the back aperture of the objective (page 47). Unless the substage is provided with centering screws, correction of the centration of the condenser is usually a task for the manufacturer.

The polarizing apparatus of the microscope is tested as indicated above.[15] The planes of vibration of the polarizer and analyzer are ascertained approximately (Experiment 1). The position of

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[14] Wright: *Jour. Wash. Acad. Sci.* 4, 301–9 (1914).

[15] See also Wright: *op. cit.* p. 61; and Johannsen: *op. cit.* p. 229.

"crossed nicols" is accurately determined (Experiment 2). The crosshairs are tested, in order that they shall represent the planes of vibration of the nicols (Experiment 3).

The accuracy of the graduations of the stage may ordinarily be assumed. If it is desired to test them, a well formed crystal of ammonium sulphate, with a long, straight edge, is placed on the stage and aligned with a crosshair. The graduations are read as accurately as possible and the crystal edge is then turned end for end and another reading is taken. All such readings, whatever part of the graduated scale is used, should differ by exactly 180°.

The graduations of the polarizer and analyzer likewise are usually accurate. They may be tested with reference to those of the stage, by crossing the nicols, aligning a crystal in the position of extinction, and reading the stage scale. Both nicols are now rotated through a given angle, and the crystal is restored to the position of extinction by rotating the stage through the same angle. The angles of rotation should check in all parts of the scales.

The crosshairs should be exactly 90° to each other; this may be checked by aligning the crystal first with one and then with the other, reading the angular rotation on the graduated stage.

OBSERVATIONS OF OPTICAL PROPERTIES BY MEANS OF THE POLARIZING MICROSCOPE

In addition to form and structure, the optical properties of transparent objects should always be investigated by means of the polarizing microscope. Even in the rare cases where the specimen has no effect upon polarized light, this negative information is of value, while in most instances a number of different optical characteristics may be observed and used in identifying the material and in interpreting its structure. In order that such observations may be of the greatest value, a clear understanding of the various phenomena is essential.

Optically Isotropic Material. — Certain substances exhibit identical optical properties in all directions, and are spoken of as optically isotropic. No matter what their orientation, they appear to have no effect upon the light which enters them, other than ordinary refraction; and between crossed nicol prisms appear dark, like the field of the microscope, whatever their orientation.

Optically Anisotropic Material. — Most crystals and many colloidal substances exhibit different optical properties in different directions, and are said to show optical anisotropy, double refraction, or birefringence.[16] The chief characteristic of optically anisotropic materials is that they possess not one index of refraction only, as in the case of isotropic substances, but exhibit different indices of refraction depending on the direction of vibration of the light passing through them.

Experiment 5. — Mount well formed crystals of strontium antimonyl tartrate in a liquid of refractive index 1.60 (or use ammonium dihydrogen phosphate in a liquid of $n = 1.49$). Illuminate with polarized light, and rotate either the crystals or the polarizer, observing the change in the shading which outlines the crystals. Determine the position of the plane of vibration of polarized light for which the refractive indices of crystal and liquid are nearly identical, as indicated by the faintness of the shading. Note that the maximum difference in refractive indices occurs at 90° from this position. Although the light is traveling in the same direction through the crystal, its direction of *vibration* governs the refractive index (Fig. 120).

Optically anisotropic substances possess the property of resolving the vibrations of light which enters them into components which vibrate only in definite, mutually perpendicular planes. These **component vibrations** (which correspond to rays of polarized light following similar paths but vibrating in different planes) travel at different rates, and therefore have different indices of refraction. The mutually perpendicular directions which correspond to the planes of vibration in an anisotropic substance are sometimes called **axes of elasticity**, or **vibration axes.**

Experiment 6. — Repeat Experiment 5, illuminating the crystals with unpolarized light, and observing the change in shading by rotating the analyzer. The refractive indices corresponding to the two components are thus manifest separately. If the analyzer is in an intermediate position, each component is partially transmitted, and their effects are superposed. If the analyzer is removed, the eye receives both sets of vibrations, and does not recognize their complex character or differentiate them from ordinary light.[17]

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[16] Such materials must not be confused with optically rotatory or optically active substances, which show entirely different properties, as described on page 287.

[17] The well-known phenomenon of a double image through a strongly doubly refractive substance such as calcite may be duplicated under the microscope with a film of sodium nitrate or *p*-dichlorbenzene crystals formed from fusion. With ordinary light, some of these, properly oriented, will give double images of dust, fissures, or inclusions in their lower surfaces, due to the marked difference in the refractive indices of the two components in the crystal.

The most striking properties of anisotropic substances are exhibited between crossed nicols, and these are the basis of the most important tests with the polarizing microscope. When a doubly refractive material is rotated between crossed nicols it appears alternately light and dark, showing "**extinction**" at 90° intervals.

Experiment 7. — Place the above preparation of crystals on the stage, between crossed nicols the planes of vibration of which are indicted by the crosshairs. Rotate the stage until a given crystal comes to the position of extinction. Note that in this position the planes of vibration of light in the crystal, as determined in Experiments 5 and 6, are parallel to the planes of vibration of the nicol prisms.

The phenomenon of extinction is exhibited whenever the planes of vibration of the specimen are in alignment with those of the crossed nicol prisms (Fig. 109), because under these conditions the polarized light from the polarizer is already vibrating in one or the other of the two planes p, p' in which all light must vibrate as it passes through the doubly refractive material. The light therefore undergoes no change in its original plane of vibration, and it is completely stopped by the analyzer. In its position of extinction an anisotropic substance gives no evidence of its doubly refractive character.

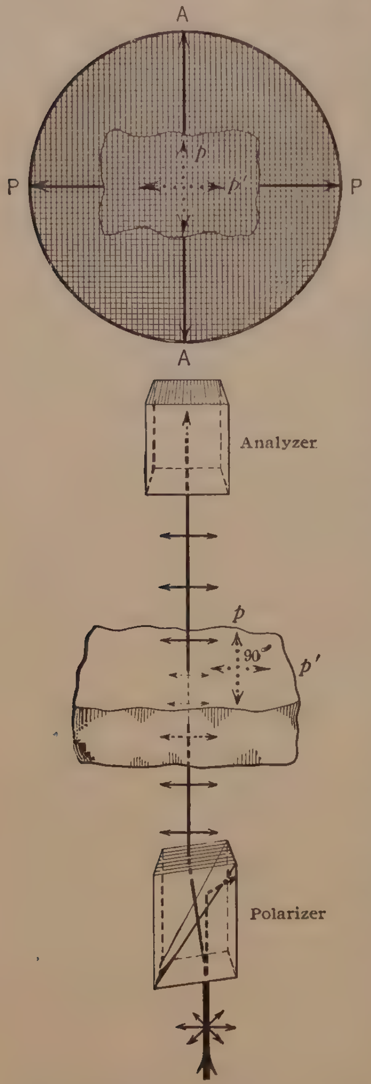

Fig. 109. Passage of Light through an Optically Anisotropic Substance between Crossed Nicol Prisms, in the Position of "Extinction."

Extinction affords a very exact means of locating the vibration directions of the specimen (pages 300, 320) and of aligning them with those of the nicols, so that the refractive index for either component may be determined (page 375). If the positions of the vibration directions of the substance are known, they may be used as a means of locating those of the nicols (page 272).

In positions intermediate between those of extinction, anisotropic materials between crossed nicols appear bright on a dark field.

Experiment 8. — Repeat the preceding experiment, noting the positions in which the specimen shows maximum brightness.

If the doubly refractive substance is in such a position that neither of its planes of vibration is in alignment with that of the polarized light which comes from the polarizer (Fig. 110), the incident vibrations are resolved into two mutually perpendicular components *c*, *c*. These component vibrations pass through the material and emerge from it, each vibrating in its own plane. Since neither of these planes is exactly "crossed" with respect to the analyzer, both component vibrations are partially transmitted by it, and the substance appears light.

The two component vibrations which pass through anisotropic material in the position of brightness travel at different velocities

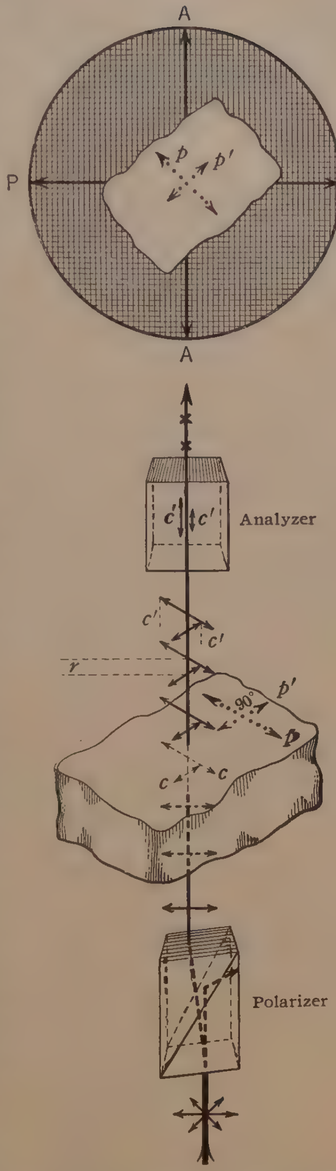

FIG. 110. Passage of Light through an Optically Anisotropic Substance between Crossed Nicol Prisms, in the Position of "Brightness."

(page 281), the slower one suffering **retardation** behind the faster. Even if the retardation r is such that the light waves are exactly "out of phase," destructive interference is impossible because the vibrations are not in the same plane. Only after a component of each, c', c', has passed through the analyzer, being thereby reduced to a common plane of vibration, is interference or reinforcement possible.

If monochromatic light is used, for certain thicknesses of the anisotropic material the retardation will be such that the two components vibrate in opposite phase after they pass through the analyzer, and interfere to produce darkness. At other thicknesses, more or less reinforcement occurs. Light of different wavelengths will suffer interference at different thicknesses of material.

Experiment 9. — Illuminate the microscope with light which is approximately monochromatic, obtained by means of a red color filter. Cross the nicols. Place a wedge-shaped piece of anisotropic material (quartz wedge, or beveled sheet of mica or selenite) in the position of maximum brightness. Slide the specimen in or out, so as to vary the thickness of the layer traversed by the light, and note that for certain thicknesses the wedge appears dark. Repeat the above procedure, using blue light, and note that the bands of darkness appear at different thicknesses, and are closer together in the wedge.

If white light is used, as is ordinarily the case, for any given retardation certain of its constituent wavelengths will be destroyed by interference. The remaining wavelengths make up the **polarization color**, which varies in hue with the amount of retardation, according to a characteristic sequence.

Experiment 10. — Repeat the preceding experiment, illuminating with white light, and note the changes in the polarization color as the thickness of the material varies. Compare the series of colors with those shown by thin films (Newton's series), and with a chart of polarization colors. Practice recognizing the position of any given color in the series, so as to be able to tell whether it corresponds to much or little retardation. Observe the successive "orders" which extend between the different reds, and learn to describe polarization colors in terms of these "orders."

Rotate the stage, and note that the *hue* of the polarization color remains constant although its *brightness* varies. Set the nicols parallel instead of crossed, and note that complementary colors are obtained.

Using a relatively thick wedge of anisotropic material (selenite, up to 0.5 mm. thick, or mica up to 0.1 mm. thick) observe the pale, "unsaturated" character of the colors in the higher orders of the series, and the grayish white which is manifest above about the fifth order ("white of high order").

Polarization colors may be used for measurement of retardation, by reference to a color chart on which the retardation corresponding to each hue is plotted.[18] For instance, " 2nd order red " indicates twice as great retardation as " 1st order red."

The **effect of thickness on retardation** has already been mentioned. The relationship is quantitative, so that retardation is directly proportional to thickness, and may be used to measure it. Slight variations in the thickness of crystals, textile fibers, or other anisotropic materials may be easily recognized by corresponding changes in the polarization colors.[19]

Experiment 11. — Examine crystals of ammonium dihydrogen phosphate which are growing or dissolving, and note the increase or decrease in the order of their polarization colors as the thickness changes.

Study the pyramidal ends of well formed crystals, and observe the series of colors on their slanting faces.

Examine fibers of viscose rayon between crossed nicols, and note the variety of first-order colors which are exhibited on a single fiber.

The **effect of relative velocities on retardation** also follows a quantitative relationship, the retardation being directly proportional to the *difference* in refractive indices for the two components in the doubly refractive material. For a given thickness, the slower component will be more retarded the greater its velocity differs from that of the faster component.

Experiment 12. — Compare the polarization colors shown by crystals of ammonium dihydrogen phosphate with those of ammonium perchlorate, using crystals of approximately the same size in each case.

Compare similarly viscose rayon with acetate rayon.

The **effect of orientation on retardation** is very marked, and a given thickness of a substance will exhibit widely varying retardations depending on the direction in which the light travels through it. This variation ranges from zero retardation (apparent isotropic character) to a maximum retardation which is characteristic of the substance.[20]

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[18] Such color charts are given by:

Johannsen: *Determination of Rock Forming Minerals* (1908).
Iddings: *Rock Minerals* (1911).
Winchell: *Elements of Optical Mineralogy* (Van Nostrand, New York, 1909. Not in later Editions.)
Michel-Lévy: *Minéraux des Roches* (Paris, 1888).
Rosenbusch-Wülfing: *Mikroskopische Physiographie* (Stuttgart, 1921–27).

The chart from the last-named work may be purchased separately from E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, Germany.

[19] Herzog makes use of the polarization colors of artificial silks to interpret their cross-sections. *Mikroskopische Untersuchung der Seide und Kunstseide* (Berlin, 1924), pp. 66–69.

Experiment 13. — Fuse some sodium nitrate on a slide, beneath a cover-glass, and press out the melt so as to form a very thin film. Allow this to crystallize, and examine between crossed nicols. Observe that although the thickness of the layer is constant (at least in adjacent crystal grains) the polarization colors vary over a wide range. Note that some grains show very low-order colors, remaining dark gray throughout an entire rotation of the stage.

Directions in which light can travel through a doubly refractive material without exhibiting the usual phenomena of extinction and retardation are called **optic axes.** Only in the case of crystal grains oriented at random, or of plate-like crystals in the tetragonal or hexagonal systems which lie flatwise, is the observation of optic axes very probable, and the double refraction of anisotropic substances is rarely overlooked because of such an orientation.

Substances in which the two components travel at markedly different velocities are said to have **strong** double refraction, while those in which the velocities are nearly identical possess **weak** double refraction. The double refraction is expressed numerically as the difference between the refractive indices for the two components. Since retardation is affected by orientation, the *maximum* double refraction for any position is taken as a characteristic constant of the material.

Addition and subtraction of retardation is possible by the superposition of two doubly refractive substances. If these are so arranged that they are both in the position of brightness, and the slower components in them are vibrating in the same plane, the effect is that of *summation* of their individual retardations. This is manifest by a polarization color which is *higher* than that of either material alone, and which actually corresponds to a retardation equal to the sum of the retardations indicated by their separate polarization colors, as determined from a color chart of the series. This might be regarded as equivalent to thickening the specimen, for the slower component in the first material is still further retarded as it passes through the second.

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[20] For this reason, only strictly axial illumination (not convergent) should be employed when precise observations of polarization colors are to be made. Otherwise the hue observed will be a mixture of the polarization colors for light passing through the substance in a variety of directions.

Experiment 14. — Examine between crossed nicols a preparation of viscose rayon in which a number of fibers cross or overlie each other. Note that where the superposed fibers are more or less parallel, the polarization color is higher than that of either. Compare the retardation corresponding to a single fiber with that shown by two acting jointly.

If, on the other hand, the two anisotropic materials are arranged so that the slower component vibrations in them are mutually *perpendicular*, the effect is that of a *subtraction* of their retardations. One might consider that the faster component in the first material is preferentially retarded by passage through the second substance so that the slower component may tend to " catch up " with it. This is evidenced by a polarization color which is of *lower* order than that of one of the specimens, and which actually corresponds to a retardation numerically equivalent to the difference of the retardations indicated by their individual polarization colors.

Experiment 15. — Examine the preparation of viscose rayon, noting the polarization colors of the single fibers, and also of the places where two of them cross at approximately right angles. Observe that the retardation of one fiber is practically neutralized by that of the other, so that little or no apparent double refraction results where they are thus superposed.

Measurement of retardation is made approximately by comparison of the polarization color with a chart but this involves recognizing the position of the color in the series of orders, and is open to the possibility of error.

By the use of **compensators** the polarization color of the specimen is compared directly with a standard, and measurement of the retardation is much more accurate. The most useful type of compensator consists of a **quartz wedge**, cut so that its thickness and retardation vary uniformly, to give polarization colors of the first three or four orders. Ordinarily the slower component vibration in the quartz is crosswise of the wedge. As the quartz wedge is inserted between crossed nicols in the 45° position, the series of polarization colors is exhibited in successively increasing orders. The variable retardation of the wedge is superposed upon that of any doubly refractive specimen which is also in the position of brightness under the microscope. If the direction of vibration of the slower component in the quartz wedge is perpendicular to that in the specimen, its retardation may be adjusted so as to compensate that of the specimen exactly.

Experiment 16. — Insert a quartz wedge between crossed nicols, and observe the series of polarization colors which are produced by varying its thickness. Determine which is the thin end of the wedge, and note the direction of this slower component vibration as marked upon the mounting.

Examine a preparation of doubly refractive material (ammonium dihydrogen phosphate or urea crystals, or viscose rayon) between crossed nicols, setting a well formed crystal or fiber in the position of maximum brightness and noting its polarization color. Now introduce the quartz wedge slowly, watching the changes of polarization color in the specimen selected. Follow the decrease in order of the polarization color of the specimen until a dark gray is produced, which marks the position of compensation. Notice that under these conditions the color in the *field* (which is due to the quartz wedge alone) is identical with that originally exhibited by the specimen. (If the polarization color of the specimen increases in order from the first insertion of the wedge, rotate the stage 90° and repeat the above operation, when compensation will be accomplished.)

Compensation of retardation is possible only when the slow components in specimen and compensator vibrate perpendicular to each other. If they vibrate in the same plane, the retardations are added, and no exact determination can be made.

When the specimen exhibits a very low order color, such as a 1st order gray, compensation is difficult because the end of the quartz wedge may not be thin enough to give the slight retardation necessary, and the point of compensation may be passed as soon as the wedge is inserted. Special compensators are manufactured for dealing with such cases; they may be provided with graduated scales for quantitative measurement of retardation through several orders.[21]

Ordinarily the range of the quartz wedge does not extend beyond the fourth or fifth order at most, so that it is of no direct value in identifying higher order colors. However, it is usually possible to find some place in the specimen thin enough to give brilliant colors which come within the range of the compensator, and thus to distinguish between " white of a high order " and " 1st order gray."

Recognition of the directions of vibration of the fast and slow components in an anisotropic material is made possible by the use of compensators. It is only necessary to orient the specimen in such a position with reference to the compensator that compensation can be obtained. Since the direction of vibration of the slower component in the compensator is known, the direction of vibration of the slower component in the specimen must be perpendicular to it for compensation to be possible.

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[21] Johannsen: *op. cit.* pp. 373–85.

The Berek compensators of Leitz are particularly designed for this purpose.

Experiment 17. — Repeat the preceding experiment, testing a number of crystals or fibers by rotating the stage until they are in positions which permit the determination of the direction of vibration of the slower component, by comparison with that of the quartz wedge.

Of the entire series of polarization colors, the greatest change in hue for a given variation in retardation occurs between the first and second orders. The purplish red located here is altered to blue by a very small increase in retardation, and to yellow if the retardation is slightly decreased; for this reason it is often called the "sensitive tint." A **"1st order red" plate**[22] made of a layer of selenite of the proper thickness, is used as a compensator having fixed retardation. It may be applied, like the quartz wedge, to the determination of the orientation of the fast and slow components in specimens which give polarization colors of the lower first order.

Experiment 18. — Insert a "1st order red" plate between crossed nicols, and note its color in the position of brightness. Ascertain the direction of vibration of the slower component from the marking on the mounting of the plate. Examine a preparation of viscose rayon or recrystallized trisodium phosphate dodecahydrate between crossed nicols, without the compensator. Note the polarization color exhibited by a typical fiber or crystal, and estimate, by comparison with a color chart, its position in the series and the corresponding numerical value of the retardation, as indicated on the chart. Now introduce the "1st order red" plate, and observe the polarization color exhibited. Note whether it is of higher or lower order than the "sensitive tint" of the compensator. Rotate the stage 90°, and again note the position of the polarization color in the series. Decide whether there is addition or subtraction of retardation, in each case, and determine the direction of the slower component in the fiber. Verify the fact that in one position the numerical retardation of the specimen is added to that of the compensator, and in the other position is subtracted from it.

The "1st order red" plate is also a very valuable means of detecting very low-order polarization colors such as would ordinarily appear as a dark gray on the darker background of the field. By its use the specimen appears in contrasting blue or yellow on a red field, and its anisotropic character, however weak, is much more strikingly evident. In the positions corresponding to extinction, the specimen appears the color of the field, red.

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[22] Also called "sensitive tint," "sensitive violet," "gypsum," "selenite," or "unit retardation" plate.

Experiment 19. — Examine a preparation of acetate rayon between crossed nicols, and note its dark 1st order gray polarization colors, which may be almost invisible. Insert the " 1st order red " plate, and notice that the fibers appear purple and orange against a red field. Determine the direction of vibration of the slower component in the fibers.

Recrystallize butter by fusion and thorough cooling, and examine with a " 1st order red " plate. Compare its appearance with the polarization colors obtained by crossed nicols alone. (See also Experiment 11, c, page 346.)

The location of the directions of vibration of the fast and slow components in any microscopic object may best be indicated on a drawing as shown in Figs. 118, 120, and 122. The specimen is first turned to the position of extinction, where its directions of vibration are parallel with those of the two nicols. These directions are noted, and the one which corresponds to the vibration of the slower component is determined by means of a compensator. If different views of the same object are available, similar observations should be made on each one, since the orientation of the vibration directions may not be the same.

In the case of markedly elongated crystals, fibers, and objects of similar shape, the relative position of the fast and slow components is sometimes described in terms of the **sign of elongation.** A substance in which the slower component vibrates approximately parallel to the long direction is said to exhibit positive (+) elongation.

The **absorption of light** in a colored anisotropic substance may vary depending on the direction of vibration, just as does the refractive index in all anisotropic materials. This phenomena is known as **pleochroism**, and is readily observed when present, by means of *one* nicol prism.

Experiment 20. — Illuminate a colored anisotropic substance (viscose rayon dyed with congo red, crystals of *o*-nitrophenol, azobenzene, iodoquinine sulphate, silver chromate, copper acetate, red ammonium picrate, or magnesium platinocyanide) with polarized light, and rotate either the nicol or the specimen. Note the color changes which take place, and the positions of the vibration direction of the illuminating beam for the two extremes of hue exhibited. Compare these positions with the two vibration directions of the substance. The experiment may be repeated, illuminating with ordinary light, and using the analyzer to separate the two differently colored components.

The pleochroic color changes exhibited by a substance may vary for different views of the specimen. If only two extremes of hue are noted, the substance is said to be dichroic; if three, trichroic. Pleochroism is best recorded by diagrams showing the directions of the differently colored vibrations in the substance. If the specimen is distinctly elongated, the **sign of absorption** may be used to express its color changes; if the light vibrating parallel to the long direction of the specimen is most strongly absorbed (darker color) the absorption is said to be positive (+).

It should be borne in mind that the absorption color of the substance may be superposed upon its polarization color, rendering difficult the recognition of "order" and even concealing double refraction completely if the specimen is intensely colored.

Optically Rotatory Material. — Certain substances possess the property of rotating the plane of vibration of polarized light which passes through them, the amount of angular rotation being proportional to the thickness of the layer traversed. This phenomenon of **optical activity** is not confined to liquids but is possessed by many inorganic and organic crystals as well. Certain substances are optically active in solution but not in the solid state, and vice versa; in some cases both crystals and liquid are optically rotatory.[23]

The rotatory effect may be manifest in isotropic material, or in substances which also exhibit double refraction. It is not ordinarily appreciable in microscopic crystals, but may be observable if their thickness is exceptionally great.

Experiment 21. — Examine a large transparent crystal (> 2 mm. thick) of sodium chlorate between crossed nicols, under low magnification. Note that the crystal appears light on the dark field, and that no extinction is observed as the stage is rotated. Turn one of the nicols to the right or the left, until the crystal appears dark. Complete darkness will not be obtained, on account of the different angular rotation for light of different colors.

Interference Figures. — The foregoing properties of anisotropic material may be observed with a relatively simple polarizing microscope, and will be found adequate for many investigations.

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[23] Tutton: *op. cit.* Chap. L.

However, the more properties which can be ascertained, the more positive the identification of materials, and the differentiation of them from similar substances. Observation of the optical properties of anisotropic substances by means of interference figures permits the determination of a number of additional characteristics, which are particularly useful in the study of crystals lacking distinctive external form.

Optical Origin of Interference Figures. — For a given thickness of doubly refractive material, the retardation varies depending on the direction in which the light is traveling through the substance (page 281). Directions in which there is no retardation or extinction are known as optic axes; either one or two such directions are possible.

Experiment 22. — Using a very low magnification, or no lenses whatsoever, examine a thick piece of colorless mica (> 0.5 mm.) between crossed nicols, tilting it in all possible directions and noting the wide range of polarization colors produced.

Instead of attempting to study all the variations in retardation in different directions through the specimen, from a series of separate observations, it is much easier to observe them simultaneously. This is accomplished by means of **conoscopic observation** between crossed nicols. By examining a lens *aperture* instead of an *image* plane, it is possible to see in the center of that aperture the optical phenomena corresponding to light traveling parallel to the axis of the system, while at the edges of the aperture the effect of oblique light may be observed (*cf*. Fig. 3). Such a pattern, summarizing the polarization colors for light traveling through a doubly refractive material in all possible directions within the angular cone of the optical system, is called an **interference figure.**

Methods of observing interference figures are based on observation of the aperture of the objective, either directly, or by means of a second compound microscope, or as it is imaged at the eyepoint. In any case, the angular cone of directions included in the interference figure is limited by the angular aperture of the condenser and of the objective.

Lasaulx' method consists of removing the eyepiece and looking directly at the back of the objective, the nicols being crossed.

Experiment 23. — Place a rather thick sheet of mica (> 0.5 mm.) in the position of brightness between crossed nicols, using an 8-mm. objective.

Remove the eyepiece, and observe the interference figure in the back aperture of the objective. If the aperture is not filled with light, employ the concave mirror, or use a condenser above the polarizer.

Repeat the observation with an objective of lower aperture (16-mm.) and with one of higher aperture (4-mm.), noting the amount of the interference figure included in each case. If the condenser is provided with a diaphragm, vary its opening and notice the effect on the extent of the figure.

Lasaulx' method gives small but sharply defined interference figures, and requires no auxiliary lenses. Directions in the figure are not inverted, and correspond to the actual directions in the *object* (not to those in the microscopic image).

Bertrand's method utilizes an auxiliary lens below the eyepiece, which functions as the objective of low-power compound microscope (Fig. 111). This second microscope system, consisting of Bertrand lens and eyepiece, is focused on the back focal plane of the objective by moving the draw-tube, and gives an enlarged image of its aperture A_2.

Experiment 24. — Examine the piece of mica as above, using a microscope with a Bertrand lens below the eyepiece. Focus the secondary microscope system by moving the draw-tube, until the interference figure appears as sharply defined as possible.

The use of the Bertrand lens gives an enlarged interference figure, but with some loss of sharpness. It permits the use of crosshairs and scales in the eyepiece. Directions in the interference figure are reversed with reference to their actual position in the object, but are not reversed as regards the microscopic image.

Klein's method. — The interference figure is observed by examining the eyepoint of the microscope, either with the naked eye, or better, with a low-power magnifier.

Experiment 25. — Study the interference figure of the piece of mica used above, by observing it at the eyepoint.

Directions in the interference figure are reversed with respect to the object, but not with respect to its microscopic image.

Special methods for observing the interference figures of small particles are very important in the examination of microscopic crystals.[24] They usually depend on the introduction of an auxiliary field diaphragm in the microscope, which serves to screen off all light except that from the part of the field occupied by the single crystal under observation.

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[24] Johannsen: *op. cit.* p. 452.

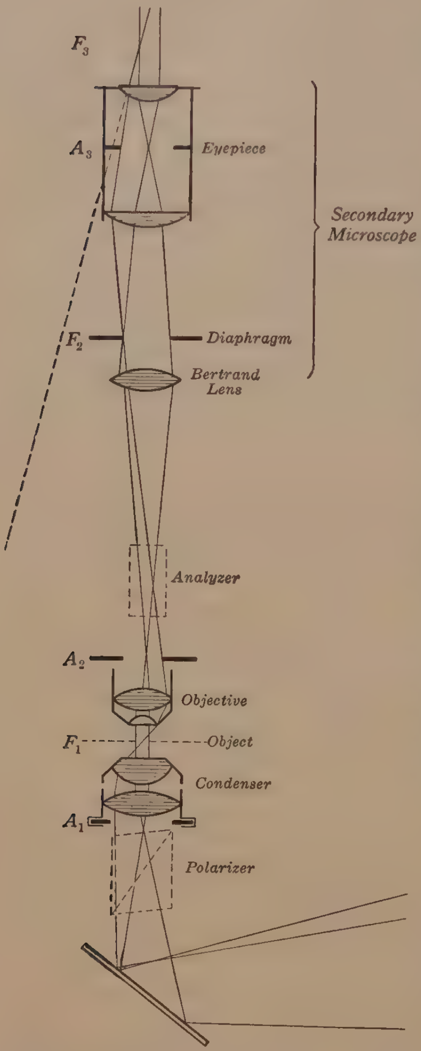

FIG. 111. Field and Aperture Diaphragms in the Conoscopic Observation of Interference Figures by Bertrand's Method.

Most of the larger crystallographic microscopes are provided with a diaphragm (F_2, Fig. 111) to vary the aperture of the Bertrand lens. This may be closed to limit the field of the microscope in conoscopic observation, and also serves to increase the sharpness of the interference figures.

If Lasaulx' method is used, a low-power magnifier mounted above an adjustable diaphragm consisting of two crossed slits is used in place of the eyepiece[25] (Fig. 23, W). This lens serves as an eyepiece and permits the desired particle to be centered and the diaphragm to be closed until it alone is visible in the field. The magnifier is then removed, and the eye placed close to the diaphragm, the interference figure being observed at the back aperture of the objective.

Any observation of interference figures of small grains of material necessitates an accurately centered stage, and very careful adjustment of the specimen in the exact center of the field. If an auxiliary diaphragm is used, it should be closed enough to insure the absence of any extraneous effects from adjacent material, without too greatly decreasing the brightness of the figure.

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[25] Wright: *op. cit.* p. 59. Apparatus obtainable from Bausch & Lomb Optical Co.

Interference figures as actually obtained from microscopic crystals are much less perfectly defined than the ideal types commonly pictured. Considerable practice is necessary in order to recognize the characteristics of interference figures which are faint or unsymmetrical, or which represent only a slight degree of retardation.

Phenomena Observed in Interference Figures. — The fact that an interference figure summarizes more or less completely the directional optical properties of the specimen renders a number of additional phenomena apparent. As the light travels through the material in nearly all directions, the variation of retardation is clearly revealed, and the optic axes, in the direction of which the retardation is zero, are indicated. It is thus possible to ascertain whether an anisotropic substance possesses one or two optic axes, and to determine other characteristics of its double refraction.

Uniaxial substances have one optic axis, along which light travels with no evidence of double refraction. If they are examined with light which passes through them in this direction only, they appear isotropic. In other directions extinction and colors are exhibited.

Experiment 26. — Recrystallize cadmium iodide or nickel sulphate from water, or iodoform from xylene, and examine the flat crystals with a 32-mm. objective, between crossed nicols. Note that they appear almost as dark as the field. Tilt the slide, or locate some crystals which are inclined, and note the polarization colors and extinction which are produced.

Select a large isolated crystal which is lying flat on the slide, and observe its interference figure, using a 4-mm. objective, and convergent light from a condenser above the polarizer. Note particularly the position and shape of the black brushes ("uniaxial cross") and the concentric rings of polarization colors in increasing order outward.

The center portion of the interference figure (O, Fig. 112) represents light traveling parallel to the axis of the microscope; it is isotropic, because the optic axis of the crystal is parallel to this direction. The arms of the cross, which are parallel to the planes of the two nicols, *P*, *A*, represent light traveling obliquely through the substance but vibrating parallel to these planes, and correspond to the four positions of extinction. The concentric zones of color, increasing in order as the obliquity of the light is increased, correspond to the positions of brightness. The greater the double refraction or the thickness of the specimen, the more orders are represented in its interference figure.

Uniaxial substances are characterized by interference figures of the above type. Even if the specimen is oriented so that its optic axis is markedly inclined to the axis of the microscope, the straight black brushes are visible successively on rotation, and from them the position of the optic axis may be inferred even if it falls somewhat outside the angular cone of the objective.

Experiment 27. — Examine the interference figures of several different crystal grains of thymol, *p*-bromphenol, or sodium nitrate, as prepared from fusion. Choose crystals large enough for only one of them to be included within the field of the objective. Note all the various appearances of partial interference figures, corresponding to different orientations of the grains, and locate the direction of the optic axis in each.

Examine similarly the interference figures of any of the following substances recrystallized from solvents: sodium nitrate, potassium trinitride, tetramethyl (or ethyl) ammonium iodide, thymol. Locate the direction of the optic axis in the interference figure, and in the object itself, bearing in mind that the microscope reverses the image of the object, and that the directions in the interference figure are also reversed if Bertrand's or Klein's method is used.

If a specimen of a uniaxial substance is so oriented that its optic axis is nearly perpendicular to the axis of the microscope, a characteristic interference figure will rarely be obtained. The direction of the optic axis may be recognized by the lower order colors in the quadrants in which it comes nearest to being included in the figure.

Experiment 28. — Examine the interference figure of crystals of ammonium dihydrogen phosphate or urea which are lying flat on the slide so their optic axes are horizontal. Note the direction of the optic axis, which is parallel to the long direction of the crystals.

The sign of double refraction of uniaxial substances depends on the relative velocities of the components vibrating parallel and perpendicular to the optic axis. The component vibrating perpendicular to the optic axis is called the **ordinary ray**, and its index of refraction is designated ω. The component vibrating in the plane of the optic axis is called the **extraordinary ray;** its refractive index varies, depending on its direction of transmission, between ω and some other extreme value designated ϵ.[26] The two refractive indices, ω and ϵ, are constants for the substance. If $\epsilon > \omega$, the material is said to be **optically positive** (+), and if $\omega > \epsilon$, **optically negative** (−).

The **determination of positive or negative character** of double refraction may be made in various ways. If the two refractive indices, ω and ϵ, have been determined (page 377) they need merely be compared. If the direction of the optic axis is known (as in the case of hexagonal and tetragonal crystals) the relative velocities of ω and ϵ may be determined by compensators.

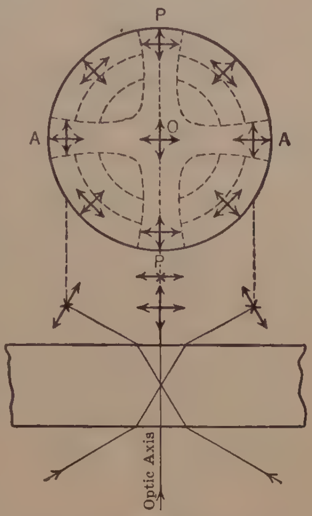

FIG. 112. Path of Rays in the Formation of a Uniaxial Interference Figure.

Experiment 29. — Ascertain the direction of vibration of the slower component in crystals of ammonium dihydrogen phosphate, by means of the quartz wedge (*cf.* Experiment 16). The optic axis is lengthwise of the crystals (*cf.* Experiment 28). Determine the sign of double refraction of the material.

Except in the cases where the symmetry of the material definitely indicates it to be in a crystal system which is uniaxial (tetragonal and hexagonal systems), an interference figure is necessary to prove its uniaxial character, on which the determination of the sign of its double refraction is based. The optic axis should be included in the interference figure, or fall just outside its angular cone.

In determining the sign of double refraction from the interference figure, it should be borne in mind that the components which vibrate "radially" in the figure are vibrating in the plane of the optic axis, and that the components which vibrate "tangentially" in the figure correspond to vibrations perpendicular to the optic axis (Fig. 112). The use of a compensator will then enable the relative velocities of the two components to be determined.

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[26] For example, the refractive indices of sodium nitrate are $\omega = 1.587$, $\epsilon = 1.336$ but if the crystal lies on a rhombohedron face (Fig. 116) the light travels through it in a direction which is not perpendicular to the optic axis, and the values $\omega = 1.587$ and $\epsilon' = 1.455$ are exhibited.

If the interference figure exhibits more than one order of polarization colors the quartz wedge is preferable as a compensator.

Experiment 30. — Examine the interference figures of any of the uniaxial substances employed in the proceding experiments, studying first a crystal which shows the optic axis parallel to the axis of the microscope (sodium nitrate, from the melt, or iodoform). Insert the quartz wedge, and note that as its retardation is increased, the concentric orders of polarization colors in the figure move inward (increase) in two opposite quadrants, and outward (decrease) in the other two quadrants. Knowing the direction of vibration of the slower component in the wedge, determine in which quadrants the slower component in the figure is parallel to it, and whether this slower component vibrates tangentially or radially. From the above information, decide the sign of double refraction.

Practice similar determinations on interference figures in which the optic axis is inclined.

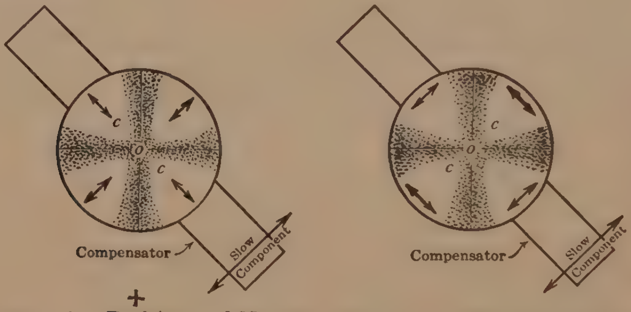

FIG. 113. Positive and Negative Uniaxial Interference Figures.

Directions of vibration of slow components indicated by arrows. *c*, *c*, quadrants in which compensation occurs. *o*, optic axis.

The appearance of the above tests may be fixed in the memory by the following scheme: in the case of an optically **positive** substance, the quadrants *c*, *c*, in which compensation occurs (colors move outward) are crosswise of the slower component in the quartz wedge, making a + sign (Fig. 113).

If only the first order of colors is visible in the interference figure, due to weak double refraction or a very thin specimen, the "1st order red" plate is preferable as a compensator.

Experiment 31. — Repeat the preceding experiment, using a "1st order red" plate, and noting the quadrants in which compensation occurs, as evidenced by patches of yellow near the point of intersection of the arms of the cross. Note that in the other quadrants patches of blue are seen, indicating that the slow component in the specimen is parallel to that in the compensator. Following the reasoning used above, determine the sign of double refraction of the specimen.

The test with the "1st order red" plate may be remembered by bearing in mind that in an optically *positive* specimen the quadrants *c*, *c*, in which compensation occurs (marked by yellow) make a + sign with the direction of vibration of the slower component in the plate (Fig. 113).

Biaxial substances possess two optic axes, along which light travels without suffering retardation, and without exhibiting the phenomenon of extinction. In other directions the usual properties of doubly refractive materials are observable. The recognition of biaxial character and its differentiation from uniaxial character is of importance in identification of materials, since all anisotropic substances fall into one or the other of these two classes. Interference figures are almost essential for this purpose, and furnish further details regarding biaxial materials.

Experiment 32. — Examine the interference figure of a sheet of mica (> 0.5 mm. thick), using a 4-mm. objective and convergent polarized light. Place the specimen in the position of maximum brightness, and note the two dark hyperbolas which are partially included in opposite quadrants of the interference figure. Rotate the stage, and observe the opening and closing of the dark "brushes," and the fact that the direction represented by the apex of each hyperbola remains dark throughout an entire revolution. Notice that the retardation increases in all directions away from the apex of each hyperbola, giving curved zones of color in increasing order.

Study similarly the interference figures of recrystallized silver nitrate, boric acid, cane sugar, ammonium persulphate, ammonium thiocyanate, potassium bioxalate, mandelic acid, malonic acid.

The typical biaxial interference figure includes dark brushes, hyperbolic in shape in the position of brightness (Fig. 114), and forming a cross if the specimen is rotated to the position of extinction. The opening and closing of the brushes, and their changing curvature, are characteristic points of difference from a uniaxial interference figure. The apex of each hyperbola *o*, *o*, corresponds to the direction of one of the optic axes, and along these directions light travels with no apparent double refraction.

Outward from the directions of the two optic axes the retardation increases, giving rise to successive curved zones of polarization colors.

The **plane of the optic axes** is easily recognized, since it is determined by their two directions, and passes vertically through a line connecting the apexes of the hyperbolas. The angle which the optic axes make with each other in this plane varies with the substance and is an important constant of it; the **optic axial angle** is designated **2 V.** The *acute* angle is measured quantitatively, and must be recognized if the sign of double refraction is to be determined from the interference figure; the direction which bisects this angle is called the **acute bisectrix** (**Bx_a**).

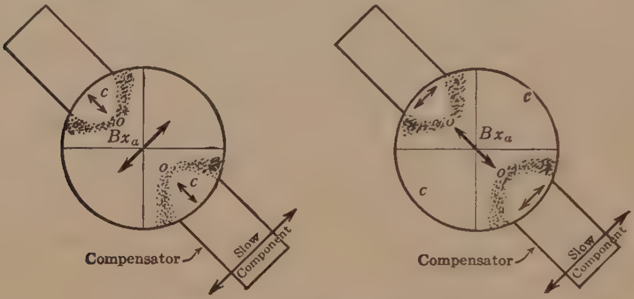

FIG. 114. Positive and Negative Biaxial Interference Figures.

Directions of vibration of slow components indicated by arrows. *c, c*, quadrants in which compensation occurs. *o, o*, optic axes. Bx_a, acute bisectrix.

If the specimen is so oriented that the acute bisectrix is not parallel to the axis of the microscope, the observation of the interference figure becomes difficult, and care must be taken to avoid wrong interpretations. At least one of the optic axes should appear in the figure, if possible.

Experiment 33. — Recrystallize sulphonal, salol, or *m*-bromnitrobenzene from the melt, in a thin film beneath a cover-glass, and examine the interference figures of a number of crystal grains in various orientations. Practice locating the optic axes and the acute bisectrix.

Dispersion of the optic axes results in their being oriented in different directions for different colors. Ordinarily this is manifest with white light as a tinge of color on either side of the black hyperbola as it passes through the optic axis. If the "brush" is bluish on its concave side, and reddish on its convex side, the optic axial angle for red light is greater than that for violet; this is expressed as $\rho > \upsilon$ or $r > v$. Various other types of dispersion are possible, but are less easily recognized in microscopic specimens.

Experiment 34. — Examine the interference figures of the following, noting the character of the dispersion of the axial angle: *p*-dichlorobenzene (from melt), ammonium thiocyanate, borax.

Recognition of the direction of the acute bisectrix is necessary if the sign of double refraction is to be determined. If both optic axes are visible in the interference figure, this is a simple matter; if one optic axis and hyperbola are visible, the acute bisectrix is located *outside* the curve of the hyperbola. If neither is visible, it is not easy to tell whether the acute or the obtuse bisectrix (Bx_0) is nearest the axis of the microscope, and a wrong determination of the sign of double refraction may be made.[27]

In ascertaining the **sign of double refraction of biaxial substances** the plane of the optic axes and the direction of the acute bisectrix must be determined from an interference figure. By means of a compensator, used in connection with the figure or with the actual image of the specimen, the direction of vibration of the slower component in the material is located. If the slower component of light traveling along the acute bisectrix vibrates crosswise of the plane of the optic axes, the double refraction is said to be **positive.** This may be kept in mind by noting that the plane of the optic axes is transverse of the slower component, making a + sign (Fig. 114). If these planes are parallel, the double refraction is negative (−). If the light is traveling parallel to the *obtuse* bisectrix, the apparent sign of double refraction, as determined by the above rule, is reversed.

If light travels through an optically positive biaxial substance perpendicular to the plane of the optic axes, the slower component will vibrate parallel to the acute bisectrix. This might be regarded as analogous to the behavior in an optically positive uniaxial substance which corresponds to the limiting case, $2\,V = 0$ (*cf.* Figs. 113 and 114).

Experiment 35. — Examine several biaxial interference figures, in different orientations, testing the direction of vibration of the slower component along the acute bisectrix, by means of a quartz wedge, and by a "1st order red" plate.

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[27] See also Winchell: *Elements of Optical Mineralogy*, Part I (1928, p. 189).

Measurement of the optic axial angle may be made approximately, by means of interference figures. The angle 2 V cannot be conveniently measured directly by means of the microscope, but the equivalent angle *in air*, 2 E, can be estimated and from it the axial angle in the specimen can be computed from the formula,

$$\sin V = \frac{\sin E}{n}$$

where n is the refractive index for light traveling along an optic axis. The refractive index need not be known exactly, since 2 E cannot be estimated with great accuracy. If the two optic axes are just included on opposite sides of the interference figure, 2 E is slightly less than the angular aperture of the objective. By using different objectives, of known angular apertures, 2 E may be estimated to a few degrees. It is convenient to have a table of the angular aperture of each objective, and of the equivalent values of 2 V for substances of refractive index 1.5 and 1.6.

Experiment 36. — Examine the interference figure of mica or ammonium sulphate, noting that 2 E is slightly less than the angular aperture of a 4-mm. objective and greater than that of an 8-mm. objective. Similarly examine the interference figure of silver nitrate, the optic axial angle of which is somewhat larger.

Either 2 V or 2 E is used as an approximate numerical characteristic of doubly refractive material, but the former is preferable if the refractive index is roughly known. If 2 E is much larger than 130° (2 V, 60–75°) both optic axes cannot be visible in the interference figure as viewed with a dry objective. If 2 V is nearly 90°, recognition of the acute bisectrix is not easy, and the sign of double refraction may be misinterpreted. If $2 E = 90°$, the substance is neither optically positive nor negative.

The optic axial angle may also be estimated from the curvature of the hyperbolas, when only one optic axis is visible in the interference figure. If the black "brushes" are sharply curved, 2 V is small; if they are nearly straight, 2 V approaches 90°.[28]

When Bertrand's method is used for determination of the optic axial angle, a micrometer eyepiece may be used to measure the distance $2\,D$ between the optic axes in the interference figure, and thus to determine $2\,E$ from the formula, $D = K \sin E$, where K is a constant[29] for the combination of lenses used which may be determined by means of one or more specimens for which $2\,E$ is known.[30]

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[28] Winchell: *op. cit.* p. 176–7.

Experiment 37. — Estimate the optic axial angle of the following substances (arranged in order of increasing $2\,V$): boric acid, lactose, urea nitrate, ammonium persulphate, potassium bioxalate, borax, ammonium sulphate, sucrose, silver nitrate, mandelic acid, sodium thiosulphate pentahydrate.

The relationship between sign of double refraction and refractive indices has already been mentioned in connection with uniaxial substances (page 292). The refractive index ω is observable for one of the two components, whatever the position of the specimen. If the light is traveling along the optic axis, the refractive index is that of the ordinary ray. The refractive index ϵ varies as the direction in which the light travels is more nearly perpendicular to the optic axis, and its highest or lowest value is observed when the light is traveling perpendicular to the optic axis and vibrating parallel to it. The magnitude of the maximum double refraction is expressed numerically as the difference between the refractive indices $\epsilon - \omega$; if $\epsilon > \omega$, it is positive (+).

In the case of biaxial substances, three characteristic refractive indices may be observed if all possible orientations are available. The highest refractive index for any component vibration is designated γ; the lowest, α. The refractive index for light traveling along an optic axis, whatever its direction of vibration, is constant and is designated as β. The maximum and minimum refractive indices are exhibited by the specimen if it is in such a position that the light travels perpendicular to the plane of the optic axes. The direction of vibration of the fastest ray, having the refractive index α, is often referred to as the X axis of elasticity; the Y and Z axes are the directions of vibration for the refractive indices β and γ, respectively.

The sign of double refraction is dependent on the relative values of the different refractive indices; if $(\gamma - \beta) > (\beta - \alpha)$, it is positive (+). The numerical value of the double refraction of the substance is expressed as $\gamma - \alpha$.

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[29] Mallard's constant. Johannsen: *op. cit.* p. 467.

[30] Ammonium sulphate, having $2\,E = 84°\,6'$, is suggested as a convenient standard for this purpose, by Fairbanks: *Amer. Mineral.* **11**, 250 (1926). $2\,V$ and $2\,E$ of a number of common chemical substances are tabulated by Tutton: *Crystallography* (1922), Vol. 2, p. 1057.

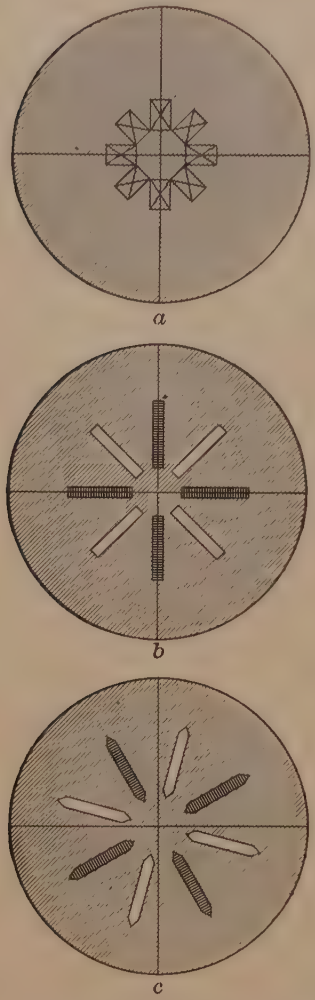

Fig. 115. Behavior of Crystals between Crossed Nicol Prisms.

a — optically isotropic.

b — optically anisotropic, parallel extinction.

c — optically anisotropic, oblique extinction.

The **optical orientation** of a substance is the arrangement of the various significant optical directions in it, with reference to its external form and structure. In the case of crystals, the orientation is usually described with respect to the crystallographic axes and prominent faces. The directions of vibration of the components in the specimen (axes of elasticity) should be ascertained, by observing its position of extinction. If the object extinguishes when its long direction is parallel to the plane of either nicol, it is said to have **parallel extinction** (Figs. 115, *b*; 118). Specimens in which the vibration directions are arranged symmetrically as shown in Fig. 116 are said to exhibit **symmetrical extinction,** which in crystals may be regarded as a special case of parallel extinction. If the directions of vibration are so oriented in the substance that extinction occurs when its long direction is oblique to either nicol, it is said to show **oblique or in-**

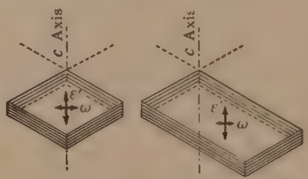

Fig. 116. Symmetrical Extinction. The vibration directions have the same angular location, irrespective of the distortion of the crystal.

clined extinction (Fig. 115, *c*). The **extinction angle** is the angle between a prominent direction (usually the long axis) of the specimen and the plane of vibration of the nearer of the two components. The maximum value is therefore 45°, and the minimum 0° (parallel extinction).

Obviously, verbal descriptions are unreliable except in the case of materials which consistently show well defined elongation in the same direction. In any case, one or more drawings are almost indispensable in presenting an adequate and intelligible record which cannot be misinterpreted (Fig. 122). The direction of the optic axis or axes and of the acute bisectrix, the planes of vibration of the fast and slow components for each of the three principal views of the specimen, and the refractive indices for each of these components, together with the absorption for each (pleochroism) should also be indicated, preferably by means of drawing (Figs. 120, 121).

The influence of external form on the observation of optical properties may sometimes be so serious as to prohibit adequate study, or vitiate any conclusions which may be drawn. The polarization of light by reflection, refraction, and diffraction at a surface becomes marked when the material is finely divided, and fine powders (such as pigments) may appear bright between crossed nicols, even if they are actually isotropic. However, individual grains will not exhibit extinction at 90° intervals, and the surface effects which render them visible may be eliminated by mounting in a medium of their own refractive index.

Optical anisotropy and its attendant phenomena cannot be readily observed if the specimen is too small. Only very strong double refraction can be detected if minute particles are under investigation; the polarization colors of weakly birefringent substances are of such a low " 1st order gray " as to be undetectable except in fairly thick layers of material. Color, refractive index, double refraction, and possibly the hues of pleochroism, may be the only optical properties observable on very tiny grains. Elongated particles may also permit the determination of their sign of elongation and absorption, and their extinction angles; very thin needles or filaments, even if of considerable length, may exhibit no more than these phenomena.

If the specimen is highly colored, or turbid, or has a very rough or fissured surface, it may be so opaque as to prevent light from passing through it, and hence its double refraction will be masked. If its refractive index is so high that its outlines are heavily shaded, only the portion which transmits light can be studied; minute grains, fine needles, or crystals of certain shapes may for this reason appear almost opaque. Immersion in a liquid of the proper index of refraction will eliminate the shadows and permit the observation of double refraction in all parts of the specimen. The shape of the object may affect the study of its interference figure by causing internal reflections and refractions which give rise to anomalous appearances. Mounting in the proper medium will minimize this difficulty.

The orientation of the material under examination is frequently limited by its shape, so that only one or two views are presented under ordinary circumstances. For instance, observations of the interference figures of fibers necessitate end views, which are not obtainable except from prepared cross-sections. Many substances crystallize in such shapes that all the crystals lie in the same position on the slide; end views of needles and prisms or edge views of plates are difficult to obtain, yet such views may be essential if interference figures are sought. It should never be assumed that all possible orientations have been observed, simply because the arrangement of the particles was left to chance. In many cases considerable search and special precautions are necessary to study the material in other than its "preferred" position. If different views are actually presented, it should be possible to recognize their relationship from their external form (Fig. 122).

Small crystals, or adjacent grains which have separated from the melt, may require special methods for the observation of their interference figures (page 289).

THE RELATION OF OPTICAL PROPERTIES TO STRUCTURE

The study of the optical properties of materials is particularly fruitful as a means of investigating their internal structure, since the specimen is studied directly, in its unaltered state, and is not dissolved or otherwise subjected to treatment which introduces various extraneous factors. The qualitative and quantitative observations which are made reveal properties which are, as combined, characteristic of the material. They may vary somewhat with variations in structure and composition, and thus serve to detect slight differences in chemical properties.

Such examinations are particularly valuable in the study of non-homogeneous specimens and mixtures, since very markedly contrasting optical characteristics may exist when chemical properties are almost indistinguishable.

Amorphous materials, such as gases, true solutions, melts, and supercooled liquids, possess no orderly internal structure and are optically alike in all directions. Consequently they are isotropic, and refractive index, dispersion, and color are their only distinctive optical properties. If they are subjected to stress, double refraction results if the internal strain is not relieved. The magnitude of this **strain double refraction** is a measure of the stress; and its sign, of the tensile or compressive character of the force acting,[31] (page 198).

In the process of recrystallization of material from the melt, supercooling may result, with marked strain double refraction, and the crystals formed may also exhibit anomalous anisotropic character due to stresses developed in cooling after solidification.[32]

Pseudo-amorphous materials,[33] such as colloidal suspensions, gels, and aggregates of ultramicroscopic micellae, behave as truly amorphous materials if the orientation of their constituent structures is wholly random. However, the action of colloidal suspensions in polarizing the light which they scatter should be borne in mind (page 230). If conditions such as applied stress or other forces tend to produce systematic arrangement of the structure, doubly refractive character results.[34]

In such a heterogeneous system, the transparency depends on the particle-size and refractive index of the dispersed phase. If the structure is practically ultramicroscopic and no great difference exists between the refractive indices of the different constituents, the specimen will be highly transparent. In the case of an aggregate of elongated or plate-like particles which consist of isotropic material, suspended in an isotropic medium, any systematic arrangement of the particles renders the system optically anisotropic. This phenomenon is known as **rod** or **plate double refraction.** It disappears if the system is rendered perfectly optically homogeneous, by permeation with a liquid of the proper refractive index, and its positive or negative character is dependent only upon the arrangement of the particles and not on whether their refractive index is above or below that of the surrounding medium.

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[31] Microscope slides which have been heated and cooled in the course of crystallization procedures may acquire marked double refraction due to strain, and thus interfere with the observation of faint anisotropic character in material resting upon them.

[32] Deodhar and Deodhar: *Phys. Rev.* **22**, 405 (1923).

[33] Friedel: Alexander's *Colloid Chemistry*, **I** (1926), p. 102.

[34] The various optical properties of anisotropic pseudo-amorphous materials are very fully discussed by Ambronn and Frey: *op. cit.*, Teil II.

See also Frey: *Kolloidchem. Beihefte* **20**, 208–43 (1924).

Rod or plate double refraction is shown by certain sols, especially if the particles are oriented by flow in the liquid (streaming anisotropy, page 231); the fine structures of diatoms may also exhibit it.

Aggregates of anisotropic particles will not exhibit double refraction unless these particles are arranged in some systematic manner, so that their effects are cumulative. Otherwise the anisotropy of each tends to compensate that of the others, and an isotropic system results. In either case, in order that the system shall be sufficiently transparent for its optical properties to be observable, it is of course necessary that the particles should be in optical contact, or surrounded by a medium of refractive index near their own. The double refraction of many substances which are commonly regarded as amorphous is due to a more or less systematic arrangement of large molecules or submicroscopic crystals. In some instances rod or plate double refraction is superposed upon this.

Pleochroism is sometimes exhibited in oriented aggregates; it is either due to the adsorption of dyes on the surfaces of material showing rod or plate double refraction, or to the systematic orientation of particles which are naturally pleochroic.[35]

Practically all the observations which can be made by means of the polarizing microscope are applicable in the study of oriented aggregates, and investigations of materials which are not obviously crystalline should nevertheless always include an examination between crossed nicols.

Perhaps the most extensive and promising field of application of the polarizing microscope, apart from the study of crystals, is in the investigation of plant and animal tissues. Characteristics, structures, and variations which are otherwise imperceptible are revealed without any alteration of the specimen, and may be observed even in living matter.[36] In addition to their importance in histology, examinations of such materials with polarized light are of direct interest to the chemist. Animal and vegetable textile fibers are all more or less doubly refractive, being essentially oriented aggregates of anisotropic micellae.[37] Not only is the polarizing microscope of value for purposes of identification[38] or differentiation between closely similar fibers,[39] but it also furnishes an excellent method of revealing strains and structural features such as irregular cross-sections,[40] the nodes and dislocations of flax and hemp,[41] or the spiral arrangement of the micellae in cotton and other fibers.[42]

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[35] Gaubert: *Comptes rendus* **149**, 1004.
Schmidt: *Mikrokosmos* **17**, 113 (1923–4).
Steidler: *Mikrochemie* **2**, 146 (1924).
Frey: *Zeits. wiss. Mikros.* **42**, 421 (1925); *Naturwiss.* **13**, 403 (1925).
Ambronn and Frey: *op. cit.*, pp. 176–185.

Double refraction is particularly significant in the study of natural and artificial fibers of cellulose, for it serves to indicate the treatment they have undergone, and affords a means of following its progress. The specific details of the process to which the cellulose has been subjected in the manufacture of a given variety of rayon affect its optical character, probably because of a greater or less completeness of orientation of cellulose micellae in the coagulation and spinning operations.[43] Nitrocellulose varies in double refraction, depending on its degree of nitration, and the sign of elongation changes from + to − at slightly less than 12 per cent nitrogen,[44] which is in the middle of the range of commercial practice. Non-uniformity of nitration, and the presence of ungelatinized fibers in solutions can be easily detected. Celluloid, cellophane, and cellulose acetate exhibit significant differences depending on their previous treatment.

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[36] An exhaustive summary of the properties of a great variety of animal tissues under the polarizing microscope is given by W. J. Schmidt: *Die Bausteine des Tierkörpers* (F. Cohen, Bonn, 1924), 528 pp. See also:

- Valentin: *Die Untersuchung der Pflanzen- und der Thiergewebe in polarisierten Lichte* (Engelmann, Leipzig, 1861), 312 pp.
- Ambronn: *Anleitung zur Benützung des Polarisations-mikroskops bei histologischen Untersuchungen* (Leipzig, 1892), 59 pp.
- Schmidt: *Zeits. wiss. Mikros.* **40**, 97 (1923); **42**, 313 (1925).
- Krause: *Enzyclopädie der mikroskopischen Technik*, Vol. III (1927), pp. 1931–38.
- Frey: *Protoplasma* **4**, 139–54 (1928).
- Schmidt: *Naturwiss.* **16**, 900–6 (1928).

[37] Katz: Hess, *Chemie der Cellulose* (Leipzig, 1928), p. 605.

[38] Krüger: *Chim. et Ind.* **10**, 126 (1923).
Mathews: *Textile Fibers* (1924), p. 337.

[39] A. Herzog: *Die Unterschiedung der Flachs und Hanffaser* (Berlin, 1926), pp. 52–55; *Die mikroskopische Untersuchung der Seide und der Kunstseide* (Berlin, 1924), pp. 61–70.

[40] Harrison: *Proc. Roy. Soc. Lond.* **94 A**, 460 (1917–8).

[41] Ambronn: *Kolloid Zeits.* **36**, 119–31 (1925).

[42] Frey: *Kolloidchem. Beihefte* **23**, 40–50 (1926).

The effect of mercerization on cotton is well defined under the polarizing microscope.[45]

The double refraction of collagen fibers in skins is similarly changed by tanning with certain materials[46] but not with others.

Starch grains, which exhibit a black cross with polarized light, have been thought to owe their anisotropic character to the concentric layers of isotropic material (a special case of plate double refraction)[47] but it has recently been shown[48] that they are actually made up of radiating crystals in the form of spherulites (page 339). The destruction of the anisotropic character is a very sensitive means of recognizing the gelatinization temperature[49] of the individual grains.

The structure of the cell walls of wood and the arrangement of the micellae in them has been studied with polarized light.[50]

Rubber develops optical anisotropy under deformation[51] and loses it on recovery. Gums, natural and synthetic resins, plastics, and other similar materials may profitably be examined with the polarizing microscope.

"Liquid Crystals" (anisotropic liquids) are not to be regarded as true crystals, but rather as aggregates of microscopic or submicroscopic doubly refractive micellae, arranged in a more or less systematic orientation as a result of mutual attractive forces.[52] The orientation of the micellae is not as symmetrical as in a true crystal, and the forces effecting it are so weak that the aggregate very readily suffers deformation when the slightest stress is applied. The chief similarity between " liquid crystals " and true crystals depends on the double refraction which they exhibit, though in some instances there is a tendency toward the formation of geometrical forms as well. In general the liquid crystalline phase of a substance is stable in a temperature range between true melting and true crystallization, as an apparent allotropic modification. Organic compounds which contain long chain structures are most likely to show a liquid crystalline form at temperatures near their melting points. The study of liquid crystals is of significance in the investigation of soaps, waxes, and similar materials of high molecular weight.

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[43] Faust: *Berichte.* **59 B**, 2919 (1926); *Cellulosechemie* **8**, 40 (1927).

[44] deMosenthal: *Jour. Soc. Chem. Ind.* **23**, 292 (1904); **26**, 443 (1907).
Ambronn: *Kolloid Zeits.* **13**, 200 (1913); *Zeits. wiss. Mikros.* **32**, 43 (1918).
Tissot: *Mem. poudres* **22**, 31 (1926).
Benedict: *Paint, Oil, Chem. Rev.* **86**, No. 7, 10 (1928).

[45] Hübner and Pope: *Jour. Soc. Chem. Ind.* **23**, 404 (1904).
Harrison: *Jour. Soc. Dyers. Col.* **31**, 198 (1915).

[46] Kuntzel: *Collegium* **1925**, 623.

[47] Gillis: *Bul. soc. chim. Belg.* **30**, 114 (1921).

[48] Alsberg and Griffing: *Proc. Soc. Exp. Biol. Med.* **23**, 728 (1926).

[49] Nyman: *Zeits. Nahr. Genussmitt.* **24**, 673.

[50] Ritter: *Ind. Eng. Chem.* **20**, 941 (1928).

[51] Kröger: *Kolloid Zeits.* **45**, 47 (1928).

A great variety of optical phenomena are shown by " liquid crystals " with the polarizing microscope. Spherulites or droplets, as well as spindles or prismatic forms, may be obtained. The fluidity of these can be demonstrated by pressing them beneath a cover-glass or otherwise stirring the preparation. The temperature ranges in which liquid crystalline modifications exist have been studied in detail by means of miscroscopes equipped with heating stages.

Experiment 31. — Dissolve some ammonium oleate in warm alcohol or kerosene, and allow to cool beneath a cover-glass. Observe the acute bipyramidal forms which separate, and test their plasticity by compression.

[52] Tutton: *Crystallography.* Vol. II (1922), Chap. LX.
Crystalline Form and Chemical Constitution (MacMillan, London, 1926), Chap. XII.
Lehmann: *Flüssige Krystalle* (Leipzig, 1904).
Methoden zur Darstellung und Untersuchung flüssiger Kristalle. Abderhalden's *Handbuch der biologischen Arbeitsmethoden,* Abt. III. Teil A. Heft 2 (Urban & Schwarzenberg, Berlin, 1922).
Vorlander: *Chemische Kristallographie der Flüssigkeiten* (Akademische Verlag, Leipzig, 1924).
Oseen: *Die anisotropen Flüssigkeiten* (Borntraeger, Berlin, 1929).
Rinne: *Crystals and the Fine Structure of Matter* (E. P. Dutton, New York, 1922), p. 63.
Tammann: *States of Aggregation* (D. Van Nostrand, New York, 1925), Chap. X.
Friedel: Alexander's *Colloid Chemistry,* I, (D. Van Nostrand, New York, 1926), p. 102.
A tabulation of many substances showing liquid crystalline modifications is given by Foote: *International Critical Tables,* Vol. I (1926), pp. 314–320.

Examine some ammonium oleate at room temperature, without recrystallization, by pressing out a thin film of it beneath a cover-glass.

Experiment 32. — Melt some cholesteryl acetate or benzoate, on a slide underneath a cover-glass, and allow it to cool slowly. Follow the formation of the liquid crystalline phase under the microscope, and observe its transformation to the truly crystalline state. Note the double refraction of each modification.

THE OPTICAL PROPERTIES OF CRYSTALS

Although the foregoing discussion of the study of optical properties by means of the polarizing microscope has been given in general terms, the most important application of the methods described is in the examination of crystalline matter. The investigation of the properties of synthetic inorganic and organic solid substances, whether as materials, intermediate stages, or ultimate products in chemical processes, involves a determination of their characteristic physical and optical properties as well as their chemical reactions. Observations of the properties of minerals, as chemical raw materials, are of similar importance, and the methods of the optical mineralogist are the basis of the procedures employed in chemical microscopy as applied to crystal studies.[53]

Crystallography, " the science of the solid state," deals with the structure and related properties of matter the atoms of which are in orderly geometrical arrangement. A crystal possesses the same internal arrangement throughout its entire extent. The three-dimensional pattern in which its atomic configuration exists is called a **space lattice**, and the forces acting within it govern the various properties of the crystal.[54] Investigations of the details of the space lattice itself are beyond the power of the microscope, and are based principally on the diffraction of X-rays by its various planes of atoms, to give diffraction patterns indicative of its internal structure. The geometrical exterior of crystals is a manifestation of their internal symmetry, but is subject to external influences and may be imperfectly developed. Examinations by means of the polarizing microscope reveal the optical properties which depend on the inner structure of the crystal and are not affected by its external form. Ideally, the microscope may be used in the observation of both geometrical and optical properties and in the correlation of them, but even when there is no external evidence of crystalline character, optical study alone may yield valuable information.

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[53] Most of the books cited on page 267 have been written from the point of view of the mineralogist, but the descriptions of properties and tests given in them apply equally well to chemical substances.

[54] In addition to the references already given, the following works give valuable discussions of the nature and properties of crystals, particularly as regards their chemical significance:

W. H. Bragg: *An Introduction to Crystal Analysis* (D. Van Nostrand, New York, 1929); *Concerning the Nature of Things* (G. Bell & Sons, London, 1925).

W. H. and W. L. Bragg: *X-Rays and Crystal Structure* (London, 1924).

Wyckoff: *The Structure of Crystals* (Chemical Catalog Co., New York, 1924).

Clark: *Applied X-Rays* (McGraw-Hill Book Co., New York, 1927), Chaps. 13, 18, 19.

Jeffries and Archer: *The Science of the Metals* (McGraw-Hill Book Co., New York, 1924).

The chief advantage of microscopic examination of crystalline material lies in the fact that the specimen is studied *as such*, rather than indirectly by means of the reactions which it may exhibit in solution. The actual solid phase present may be investigated directly, rather than merely the ions which it yields when dissolved. Identifications based wholly upon optical tests have been used for many years by mineralogists, and are frequently possible in chemical work. If chemical information is employed in conjunction with them, substances may be even more easily and positively characterized. Furthermore, the close connection between optical and other physical properties is particularly valuable in recognition of allotropy, while the marked differences often existing between substances that are chemically very similar serve to facilitate distinguishing isomers and homologues.

Geometrical Properties of Crystals. — **Symmetry** is the most important characteristic of crystal form; it may be defined as the number and arrangement of like directions, and is externally manifest by the plane faces which bound most well developed crystals. The orientation of these faces with respect to each other is of much greater significance than their size or shape; the angles at which the faces intersect are constant for any given substance. The distance of a face from the center of the crystal which it bounds depends solely upon the number of layers of atoms deposited in planes parallel to it in the space lattice, and not upon any alteration of spacing in the structure. It may therefore be said that moving a crystal face parallel to itself does not alter its crystallographic significance, although apparently the external symmetry of the crystal may be destroyed thereby. The faces of the crystal are an expression of its symmetry only when they are able to develop freely in all directions; the fact that they may be prevented from doing so by external conditions does not affect the structure of the space lattice itself, which is the fundamental characteristic of the crystal. For instance, a growing cube of sodium chloride is no less a crystallographic cube because one of its faces encounters the surface of the mother liquor and cannot extend above it while on the other faces material is further deposited, resulting in a flattened rectangular parallelopiped. The spacing of atoms of sodium and chlorine is the same in the three coordinate directions, and this is the real criterion of cubic character (Fig. 131).

On the basis of the angular arrangement of the planes or faces exhibited by a crystal, certain directions through it may be recognized as **axes of symmetry**. Rotation about these axes results in a recurrence of arrangement two, three, four, six, or more times in a revolution. **Planes of symmetry**, on either side of which the crystallographic structures are symmetrical as mirror images, may also be observed. The symmetry of the crystal with respect to these axes and planes determines its **crystal system**, and its class within that system.

The principal axes of symmetry which serve to characterize the common properties of all the crystals belonging to a given system are called **crystallographic axes.** They may be arranged at right angles to each other, or inclined, and the spacing of atoms in these different directions may be identical, or may vary. Like axes are designated by *a*, a unique axis by *b*, and unlike axes by *a*, *b*, and *c*.[55] The **axial ratio** gives the relative magnitudes of the different atomic spacings; it is calculated from measurements of the slopes of crystal faces not perpendicular to an axis, and preferably also from X-ray studies of the space lattice.

The terminology used in designating various crystal faces and planes is described in the references given above. The "Miller indices" are most commonly employed; they consist of the reciprocals (reduced to the simplest possible whole numbers) of the intercepts of the crystallographic plane in question with the crystallographic axes. These intercepts are given in the order of the *a*, *b*, and *c* axes; thus 111 indicates an octahedron face, and 001 a plane perpendicular to the *c* axis (*cf.* Fig. 122).

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[55] It should be emphasized that a crystallographic axis is a *direction,* and not a line which passes through a crystal in a given place.

Crystal faces which are alike constitute a **form**: several such forms may be combined, though ordinarily only a few are represented in the case of a microscopic crystal. When a crystal grows freely in all directions the forms which it exhibits, represented by faces of different shapes and sizes, constitute its **habit**. The habit may be greatly modified by external conditions, producing distorted crystals or causing the suppression of certain forms and increased development of others (page 337). Ordinarily, the optical properties of a crystal are related only to its principal symmetry and rarely have any connection with the minor details of symmetry.

Geometrical and Optical Characteristics of Crystal Systems. — The optical properties of crystals in the various systems may best be kept in mind by considering them in connection with the geometrical characteristics of each system, according to the following summary. The examples given are chosen as particularly typical of the various systems, and may advantageously be studied in detail.

Cubic System: (*Isometric; Regular; Tesseral*). — Arrangement of atoms according to a rectangular pattern, with respect to three crystallographic axes which are mutually perpendicular; spacing of atoms alike in all three directions.

Cube, octahedron, tetrahedron, rhombic dodecahedron, and combinations of these forms, are most common. Crystals equidimensional, unless growth in certain directions is restricted by external conditions. Octahedron and tetrahedron often show trigonal or hexagonal symmetry, in certain positions (Fig. 79).

Optically isotropic, since all orientations are alike. A single refractive index, independent of direction of vibration or transmission of light.

Examples: *Cube* — sodium or potassium chlorides, bromides, and iodides; sodium chlorate and bromate.

Octahedron — alums; strontium, barium and lead nitrates; ammonium and potassium chloroplatinates; silver chloride; arsenic trioxide.

Tetrahedron — sodium uranyl acetate.

Rhombic dodecahedron — hexamethylene tetramine.

Pentagonal dodecahedron — cerium formate.

Tetragonal System: (*Quadratic*). — Arrangement of atoms according to a rectangular pattern, with respect to three crystallographic axes which are mutually perpendicular. Spacing of atoms alike in the direction of two of the axes (*a* axes), and either greater or less in the direction of the other, unique axis (*c* axis).

Bipyramids (consisting of acute or obtuse "octahedra"), more or less elongated prisms which are rectangular in cross-section, and combinations of these forms are most common. Four-fold symmetry is commonly exhibited by "end views" and two-fold by "side views."

Optically anisotropic, with maximum double refraction exhibited when light travels through the crystals perpendicular to the unique axis, since in this position the different spacing of atoms in different directions is manifest. Vibration directions in crystal parallel to a and c axes, as consistent with its symmetry (Fig. 118). Parallel or symmetrical extinction exhibited by "side views" of crystals. Symmetrical extinction shown by crystals lying on pyramid faces.

Uniaxial. — Optic axis parallel to c axis; crystals appear isotropic if light travels through them in this direction, since in this position the like spacings of atoms are manifest. Bipyramids and prisms appear isotropic only if viewed "endwise." Interference figures observable only in or near this orientation.

Two principal refractive indices, exhibited when light is traveling through the crystal perpendicular to the c axis: ϵ for vibrations parallel to c, and ω for vibrations perpendicular to c. Isotropic views give refractive index ω only. No relationship between numerical values of ϵ and ω and spacing of atoms in different directions.

Colored substances may show pleochroism.

Examples: *Bipyramid* — nickel sulphate hexahydrate, potassium trinitride, beryllium sulphate tetrahydrate, *p*-bromphenol.

Prism and bipyramid — potassium or ammonium dihydrogen phosphates and arsenates; potassium or ammonium copper chloride dihydrate, mercuric cyanide, tetramethyl ammonium iodide, guanidine carbonate, urea.

Hexagonal System. — Arrangement of atoms according to a hexagonal or trigonal pattern, with respect to three like crystallographic axes at 120° to each other, and a fourth (c) axis perpendicular to the plane of the a axes. Spacing of atoms alike in the directions of the three a axes, and greater or less in the direction of the unique c axis.

The hexagonal system is frequently divided into two main classes, hexagonal and trigonal, of which only the most distinctive microscopic features are given.

Hexagonal class: Six-sided pyramidal forms, and prisms, singly or in combination; very short prisms truncated by pinacoid planes to give hexagonal plates. Needle or plate-like habit most common; equidimensional crystals rare. Six-fold symmetry exhibited by "end views;" two-fold symmetry by "side views."

Trigonal class: Rhombohedra, bounded by six rhomb-shaped faces, and ideally forming equidimensional crystals. Three-fold symmetry exhibited by "end views" (rare); planes of symmetry exhibited by side views of rhombohedra.

Optically anisotropic, with maximum double refraction when light travels through the crystals perpendicular to the c axis, since in this position the different spacing of atoms in different directions is manifest. Vibration directions in crystal parallel to a and c axes, as consistent with its symmetry. Parallel extinction exhibited by side views of prisms or plates (Fig. 120);

symmetrical extinction exhibited by crystals lying on pyramid or rhombohedron faces (Fig. 116).

Uniaxial. — Optic axis parallel to *c* axis; crystals appear isotropic if light travels through them in this direction, since in this position the like spacings of atoms are manifest. Plates lying flatwise, and prisms on end (rare) appear isotropic. Isotropic views of pyramids and rhombohedra are uncommon. Interference figures are obtainable from plates, rhombohedra, and pyramids nearly on end.

Two principal refractive indices, exhibited when light is traveling through the crystal perpendicular to the *c* axis: ϵ for vibrations parallel to *c* and ω for vibrations perpendicular to *c*. Isotropic views show refractive index ω only. Crystals lying on a rhombohedral face give ω for vibrations transverse of their plane of symmetry, and a value between ϵ and ω for vibrations in this plane (page 293).

Colored substances may show pleochroism.

Examples: *Prism* — normal sodium phosphate dodecahydrate, strontium chloride hexahydrate, trimethyl (or ethyl) ammonium chloride or bromide.

Prism and pinacoid (plates) — iodoform, lead iodide, cadmium iodide.

Prism and pyramid — strontium antimonyl tartrate.

Rhombohedron — sodium nitrate, calcium carbonate, thymol.

Prism and rhombohedron — acetamid.

Orthorhombic System (*Rhombic; Trimetric*). — Arrangement of atoms according to a rectangular pattern, with respect to three unlike crystallographic axes, mutually perpendicular to each other. Spacing of atoms different in the direction of each of the axes, of which the shortest is commonly designated *a*, the longest *c*, and the intermediate *b*.

Rectangular parallelopipeds, which are bounded by pinacoidal faces; prisms; rhomb-shaped tablets; pyramidal forms; combinations of forms common, giving many-faced crystals. Two-fold symmetry commonly exhibited by principal views, with three planes of symmetry usually present.

Optically anisotropic, with double refraction exhibited when light travels through the crystal in any direction not parallel to one of its optic axes. Vibration directions in crystal parallel to *a*, *b*, and *c* axes, as consistent with its rectangular symmetry. Parallel or symmetrical extinction exhibited by all three principal "views," with different birefringence and pleochroism for each.

Biaxial. — Two optic axes, not parallel to the crystallographic axes but lying in the plane of two of them with which the acute and obtuse bisectrices are parallel. Directions of optic axes otherwise unrelated to symmetry of crystal, and rarely perpendicular to any crystal face. Interference figures often not readily observable unless crystals are oriented at random, and all possible views are examined.

Three principal refractive indices, for vibrations parallel to the three crystallographic axes: γ, highest; β, intermediate; α, lowest. Only two of these exhibited in any one view of the crystal, with intermediate values in all orientations except when light travels through the crystal parallel to a crystallographic axis. Refractive index β shown if the direction of light is parallel to an optic axis. No relationship between numerical values of γ, β, and α, and spacing of atoms in different directions.

Colored substances may show pleochroism, with different hues for different orientations.

Examples: *Prisms* (lying on a pinacoidal face) — ammonium and potassium sulphates and chromates, potassium nitrate.

Prisms (lying on a prism face) — ammonium perchlorate, ammonium magnesium phosphate hexahydrate.

" *Rhombs* " — silver sulphate.

" *Rectangular tablets* " — silver nitrate.

Monoclinic System (*Clinorhombic; Monosymmetric; Oblique*). — Arrangement of atoms according to a rectangular pattern in two planes and an oblique pattern in the third, with respect to three unlike crystallographic axes, two of which are mutually perpendicular to a third one but not to each other. Spacing of atoms different in the direction of each of the axes, of which the longer inclined one is designated c and the shorter a; the b axis is perpendicular to these. The acute angle between the a and c axes is called β. Only one plane of symmetry, that of the a and c axes; oblique symmetry in the other principal views.

Combinations of forms common (Fig. 122), and often similar to those exhibited by orthorhombic crystals except for the oblique symmetry. Obliquity of structure sometimes not evident except from angular measurements, if β is nearly 90°.

Optically anistropic, with double refraction exhibited when light travels through the crystal in any direction not parallel to one of its optic axes. Vibration directions in crystal parallel to b axis, and to plane of a and c axes, since rectangular symmetry is exhibited under these circumstances; the location of the vibration axes in the plane of symmetry is wholly unrelated to the obliquity of the space lattice, but in general neither will be parallel to the a or c axis. Parallel or symmetrical extinction exhibited by two principal views of the crystals. Oblique extinction shown in any orientation in which light does not travel in the plane of symmetry; maximum obliquity for rays perpendicular to this plane.[56] Different birefringence and pleochroism for each different principal " view."

Biaxial. — Two optic axes, not parallel to the crystallographic axes nor necessarily lying in the plane of any two of them. Directions of optic axes symmetrical with respect to the plane of symmetry of the crystal, but otherwise unrelated to it, and rarely perpendicular to any crystal face. Interference figures often not readily observable unless crystals are oriented at random, and all possible views are examined.

Three principal refractive indices, two for mutually perpendicular vibrations in the plane of a and c, and the other for vibrations parallel to b. Otherwise like orthorhombic crystals.

Examples: *Prisms, plane of symmetry lengthwise* (parallel and oblique extinction) — ammonium sodium hydrogen phosphate tetrahydrate, strychnine nitrate, *p*-brombenzoic acid, *o*-nitrophenol, oxalic acid dihydrate, pyrocatechol.

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[56] Since the oblique symmetry and extinction of monoclinic crystals are manifest only in one of the principal " views," it is sometimes difficult to distinguish them from those of the orthorhombic system.

Prisms, plane of symmetry crosswise (parallel extinction only) — sodium sulphate decahydrate, ammonium tartrate.

Tablets, flattened parallel to plane of symmetry (oblique extinction most common) — barium chloride dihydrate, calcium sulphate dihydrate, sodium thiosulphate pentahydrate.

Tablets, flattened perpendicular to plane of symmetry (parallel or symmetrical extinction most common) — potassium chlorate, borax, cupric acetate monohydrate, ammonium persulphate, ammonium ferrous sulphate hexahydrate, ferrous sulphate heptahydrate, sodium benzene sulphonate, urea nitrate, mandelic acid, potassium bioxalate.

Triclinic System (*Asymmetric; Anorthic*). — Arrangement of atoms according to a pattern oblique in three directions, with respect to three unlike crystallographic axes mutually inclined to each other. Spacing of atoms different in the direction of each of the axes, which are designated a, b, and c and intersect at angles α, β, and γ. In most crystals, several different locations of these axes are possible, so that descriptions based on them often appear inconsistent unless the orientation is known. No planes or axes of symmetry present; oblique symmetry in all principal "views" of the crystal.

Combinations of forms common, and recognition of similar faces difficult unless angles are measured.

Optically anisotropic, with double refraction exhibited when light travels through the crystal in any direction not parallel to one of its optic axes. Vibration directions in crystal oblique to crystallographic axes, since no planes of symmetry exist; no connection between their orientation and the obliquity of the space lattice. Oblique extinction shown by all views, the angle being different with each. Birefringence and pleochroism also differ with each view of the crystal.

Biaxial. — Two optic axes, oriented in positions unrelated to the crystallographic axes, and rarely perpendicular to a crystal face. Interference figures often not readily observable unless crystals are oriented at random, and all possible views are examined.

Three principal refractive indices, for vibrations mutually perpendicular to each other.

Examples: *Tablets* — potassium bichromate, boric acid, cupric sulphate pentahydrate, potassium persulphate, malonic acid.

Microscopic Examination of Crystalline Material. — Systematic determination of the properties of chemical crystals involves application of the optical tests which have already been described, and study of the geometrical and physico-chemical characteristics of the material. The general procedure is similar to that of the mineralogist, but ordinarily the chemist can carry his observations much further on account of being able to recrystallize his material under the microscope.[57] If the material cannot be recrystallized in form suitable for study, on account of its insolubility, or for other reasons, a fairly complete investigation of its optical properties is nevertheless possible. All the observations and determinations described below, with the exception of the various details of optical orientation and geometrical exterior, may be made upon fragmental material. Large crystals or aggregates may be crushed to about 100 mesh or coarser and the individual grains studied, according to the methods used in the study of powdered minerals.[58]

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[57] Various procedures for the recrystallization of specimens for study are given in Chapter X.

The sample to be examined should be studied first at low magnification, just as received. In this way its original crystal character may be noted, even if the forms are far from perfect, and may be employed in interpreting its history. Furthermore, many slightly soluble materials are better crystallized on a large scale than in a drop under the microscope, and should not be dissolved without their initial character being utilized fully. Efflorescence or other alteration of the sample should be noted before it is brought into solution, and it may be desirable to mount the material in an inert liquid in order to increase its transparency and to render any heterogeneity or foreign matter visible. Such procedure is particularly useful in the study of commercial chemicals in powder form, where different hydrates and other mixtures of compounds may be present. Optical tests may be applied to the original specimen, if desired.

If recrystallization is to be made from solution, the dissolving of the crystals should be followed under the microscope. In this way hydration, decomposition of double salts, hydrolysis, and similar phenomena may be noted, and the solubility of the material roughly gaged. If acids, alkalies, or salt solutions are used as solvents, their action should be similarly followed. The behavior of the sample on heating should always be noted; efflorescence, decomposition, transformations, and the approximate melting point may be indicated.

As the crystals form from solution or from the melt, their growth should be observed under the microscope, since different hydrates or allotropic modifications may appear, and the form of the crystals may show variation with time, temperature, and other conditions.

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[58] In addition to the works already mentioned the following may be cited as dealing primarily with the study of pulverized materials and isolated crystals rather than with thin sections:

Larsen: Microscopic Determination of the Non-Opaque Minerals, *U. S. Geol. Survey, Bull.* **679** (1921).

Fry: Tables for the Microscopic Identification of Inorganic Salts, *U. S. Dept. Agr. Bull.* **1108** (1922).

Insley: *Jour. Amer. Ceram. Soc.* **11,** 812–28 (1928).

The **geometrical form** of the crystals obtained should be studied with great care, every effort being made to ascertain their three-dimensional structure according to the methods given on pages 76, 175. Particular pains should be taken in the examination of crystals which lie in different orientations and present different "views" and different optical properties. By correlating the observations on crystals in more than one position their true symmetry is established and invaluable checks are afforded in working out their optical orientation. Simple crystals with few and well formed faces are preferable for study, since they are less likely to be confusing even if somewhat distorted (Figs. 79, 116). Ordinarily, only typical crystals which can be found in quantity in any preparation are of value; malformed individuals, fragments, or aggregates are misleading, and are too rarely duplicated to be characteristic.

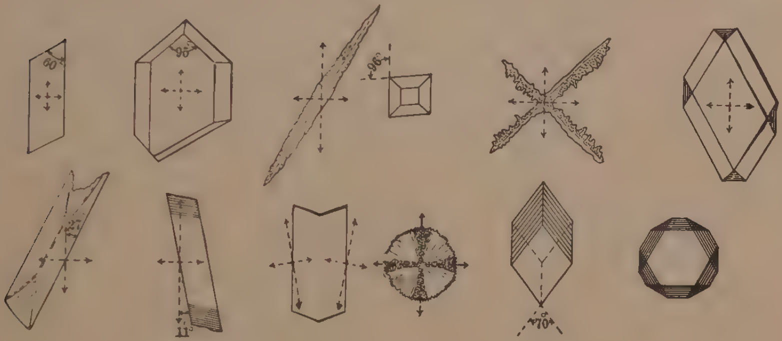

Fig. 117. Note Book Sketches of Crystals.

Accurately made **drawings** of a number of representative crystals are an essential part of the study. These should show different views, as an aid in interpreting the three-dimensional form (Figs. 117, 118, 122). The commoner variations in proportions should be represented, especially if the crystals tend to grow flattened against the slide; the relationship of these modifications of habit may be clearly indicated by marking corresponding angles and faces. In recording the crystal forms exhibited by any given substance, their actual appearances, as seen lying on the object slide, should be noted. "Perspective" drawings (clinographic projections) of ideal crystals, such as those to which conventional crystallographic descriptions are too often limited, are sometimes valuable as an aid in visualizing the shape as a geometrical solid, but they should never be employed as a substitute for drawings which picture the forms and views most commonly encountered in microscopic preparations. The appearance of a substance is often so distinctive that it can be recognized by its shape alone, without the need of optical or chemical tests; experience in the study of known samples, especially those likely to occur in the investigations at hand, may render such identifications possible at a glance.

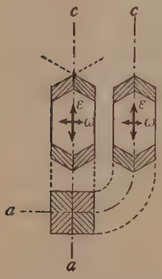

Fig. 118. Three "Views" of a Tetragonal Crystal, showing manner of indicating vibration directions. $\epsilon < \omega$, therefore the crystal is optically negative.

In constructing drawings of crystals, parallelism or obliquity of faces and edges, the direction of slope of surfaces inclined to the axis of the microscope, the presence of axes or planes of symmetry — all should be carefully noted as aids in determining the crystal system to which the material belongs. The Miller symbols for the faces may be employed if there is no question as to the directions of the *a*, *b*, and *c* axes; it is unwise to use them as a substitute for drawings, since the location of these axes is to a considerable extent arbitrary and one or more alternative locations are often possible. If the symbols are used, it is customary to list the faces in the order of their relative areas.

Measurements of prominent angles (page 425) should be made on several crystals, and indicated on the drawings (Figs. 117, 122). Ordinary crystallographic descriptions include only interfacial angles as measured by a goniometer; in addition, characteristic angles between edges or between an edge and a crystal face are easily determined microscopically and are often more directly useful in the recognition of symmetry. If interfacial angles not readily measurable under the microscope (page 426) are desired for comparison with published data or for the computation of approximate axial ratios, their values may be calculated from microscopic measurements of other angles. The following formulas will be found useful for such purposes (see Fig. 119):

$$(1)\quad \tan\frac{\sigma}{2} = \frac{\tan\phi/2}{\cos\theta}$$

$$(2)\quad \sin\frac{\sigma}{2} = \frac{\sin\phi/2}{\cos\tau}$$

$$(3)\quad \cos\theta = \frac{\cos\tau}{\cos\phi/2}$$

$$(4)\quad \tan\theta = \tan\tau\cdot\cos\frac{\sigma}{2}.$$

Particularly in the examination of distorted crystals a few comparisons of angles will indicate whether similar or different "views" are exhibited and will prevent mistaking symmetrical for oblique extinction (Fig. 116). Considerable experience and care are necessary to avoid being misled by malformed crystals.

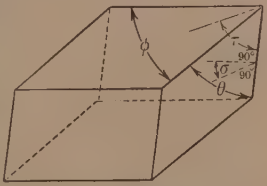

Fig. 119. Plane and Interfacial Angles.

Cleavage, zones of inclusions, etch-figures, turbidity, color, and similar properties ought also be noted, as more or less characteristic of the material.

The relative **refractive index** of the crystals, as compared with that of their mother liquor or melt, will be indicated by the intensity of the shading which surrounds them. In almost all cases the crystals are more refractive than the liquid from which they separate. The index of refraction may be determined quantitatively if desired (page 365). In the case of doubly refractive substances two refractive indices should be obtained for each of the principal "views," unless ϵ, or α, β, and γ are directly obtainable (page 375); the values may be noted on the drawings as shown in Fig. 120.

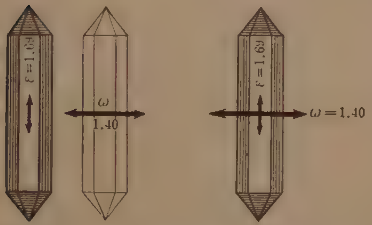

Fig. 120. Hexagonal Crystal, showing method of indicating refractive indices of anisotropic material. $\epsilon > \omega$, therefore the crystal is optically positive.

Pleochroism, when present, may be indicated on the drawings as shown in Fig. 121, for each different "view" of the crystals.

The **strength of double refraction** may be qualitatively estimated from the order of the polarization colors, the thickness of the crystals being also considered (page 281). Strong double refraction, as manifest by change of shading when one nicol is used and the stage rotated, is worth noting. The numerical values of the refractive indices afford a more exact means of recording the strength of double refraction (page 282).

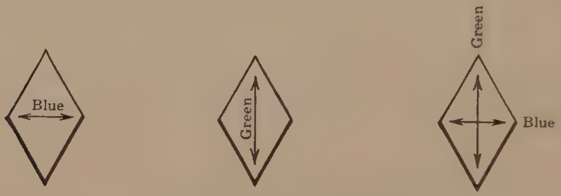

Fig. 121. Methods of Indicating Pleochroism.

The **positions of extinction** should be determined carefully for the different "views" of doubly refractive crystals. Only well formed crystals with practically perfect edges and faces should be chosen for study. A low-power objective and strictly axial rather than convergent illumination are preferable. Focusing must be very exact, to avoid spurious shading or error due to faces lying in other planes.

The crystal is centered, and placed so that the edge or direction selected for reference is exactly parallel to one of the crosshairs, and the graduations of the stage are read. The crystal is then turned to the nearest position of extinction, by rotating the stage, and the graduations are read again, the difference being the **extinction angle.** Measurements should be repeated, and should be made on several crystals, as checks on the accuracy of the determination. The actual angle measured should be indicated on the drawing of the crystal or referred very definitely to a characteristic crystal face. The simplest procedure is to draw the position of the planes of the nicols (as represented by the crosshairs) when the crystal is in the position of extinction (Figs. 117, 118, and 122).

The **directions of vibration of the fast and slow components** in an optically anisotropic crystal should be determined for each of its principal "views." Their angular position may be measured as above, and by means of "compensators" (page 284) such as a "1st order red" plate or a quartz wedge their relative velocities may be ascertained. The orientation of the vibration directions in the crystal is best represented by crossed arrows (Figs. 120 and 122) the longer of which corresponds to the faster component (lower refractive index).

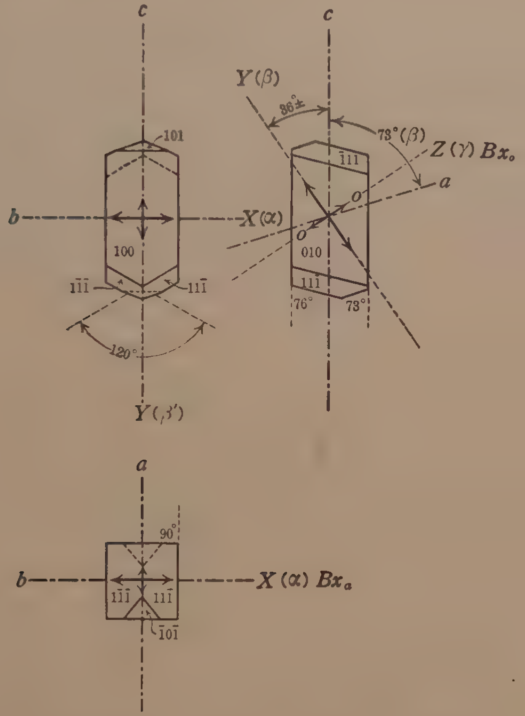

FIG. 122. Method of Indicating Optical Orientation.

In the case of consistently elongated crystals, the sign of elongation (page 286) may be recorded, and in tetragonal or hexagonal crystals the sign of double refraction (page 292) may be determined from the above observations.

Interference figures should be studied if obtainable (page 289), in order to gain information as to the uniaxial or biaxial character and the sign of double refraction of the crystals. The magnitude of the optic axial angle, the dispersion of the optic axes, and their directions in the crystal ought also to be observed and recorded. Ordinarily, in searching for an interference figure of a granular material, such as a recrystallized salt, a large number of particles should be examined between crossed nicols under a moderate magnification. Any which show particularly low order colors for their thickness, and which do not extinguish sharply when the stage is rotated, should be tested further with conoscopic observation. A number of trials may be necessary in order to find crystals which give clean-cut figures of unmistakable characteristics. If the material tends to lie in a single orientation, due to elongated or platy habit, it is often effective to suspend the grains at random in a viscous liquid such as Canada balsam. If the original sample is coarse-grained, powdered fragments of it may be used for this purpose, though their optical orientation cannot be obtained unless they show geometrical form. Substances which are easily recrystallized by fusion usually afford good interference figures if allowed to cool slowly. The crystal grains with lowest order colors should be examined.

The **optical orientation**, which summarizes the above properties of doubly refractive crystals, should be represented somewhat as shown in Fig. 122. Verbal descriptions are likely to be misleading unless the positions of the crystallographic axes are known. For this reason the optical orientation should be referred to the habit of the crystals actually obtained by growth on a microscope slide, as well as to the forms of ideal crystals. Ground material. or crystals in aggregates as grown from the melt may not permit such observations, but if definite faces are present the position of some or all of the important optical directions can always be reported. Even incomplete data on crystal form and optical properties are multiplied in value by relating them to each other in terms of the optical orientation of the material.

The **terminology of crystallographic descriptions** includes a number of symbols which are more or less standard but which must be used with exactness and caution in order to avoid being misleading or confusing. As an example of their application, the optical orientation of the monoclinic crystal of borax shown in Fig. 122 may be taken as typical.

The *b* axis is of necessity perpendicular to the plane of symmetry of the crystals; the *a* and *c* axes are inclined to each other. Their positions have been chosen in accordance with crystallographic procedure, c being parallel to the long direction and a parallel to the face 001, in order that the angle between them, $a \wedge c$ (or $100 \wedge 101$), can be measured by means of a goniometer to give the acute angle $\beta = 73°$ The Miller indices (page 310) with reference to these axes are shown, and serve to designate the angles between the faces, thus: $100 \wedge 101 = 73°$; $100 \wedge 010 = 90°$, etc. The angle $010 \wedge \bar{1}11$ cannot be readily measured under the microscope, for the crystals will not lie in such positions that the edge between these faces is parallel to the axis of the microscope. The angle of 76° between 100 and the edge in which $\bar{1}11$ and its symmetrical face intersect is characteristic and easily measured microscopically, but cannot be directly determined by a goniometer; the terminal angle of 120°, as measured in the plane of 100, is also distinctive. The habit of the crystals is ideally as shown, but flattening parallel to 100 is common and this view occurs most commonly in microscopic preparations; much shorter prisms are also frequent. Occasionally crystals are found growing on 101 or 111 faces, with marked flattening parallel to these planes; forms lying on the edge $100 \wedge 010$ are also encountered.

As viewed endwise or $\perp$ 100 the crystals exhibit parallel or symmetrical extinction, with the lower refractive index ($\alpha = 1.447$) for vibrations crosswise (faster component). The refractive index for vibrations in the plane of symmetry ($\parallel$ 010) varies between 1.470 and 1.472, depending on the direction which the light travels in this plane. The crystals as seen $\perp$ 100 present the highest double refraction of any of the principal views, and give an interference figure indicating that the plane of the optic axes lies crosswise of the crystals.

As viewed $\perp$ 010, the oblique structure of the crystals is manifest by oblique extinction, the faster component making an angle of about 36° with the c axis; the extinction angle varies with the wavelength of light (dispersed extinction). The double refraction of this view of the crystals is low, the refractive indices being $\gamma = 1.472$; $\beta = 1.470$. Interference figures which show both optic axes symmetrically are readily obtained, hence the acute bisectrix Bx_a is parallel to b. The plane of the optic axes o,o is parallel to the direction of the slower component, hence the sign of double refraction is negative ($-$). The optic axial angle $2\,V =$ *circa* 39°; $2\,E =$ *circa* 59°; the values are greater for red than for violet light ($\rho > v$) and the dispersion of the optic axes is "crossed," as is consistent with the dispersed extinction exhibited. The positions of the vibration axes X, Y, and Z are determined by the directions of vibration of α, β, and γ, to which they are respectively parallel. They are used to describe the optical orientation, thus: $X = b = Bx_a$. $Y \wedge c =$ *circa* 36° (= the extinction angle). $Z \wedge c = 90° - 36° =$ *circa* 54°; $Z = Bx_o$. Ax. pl. $\parallel Z$, $\perp$. Y.

A number of descriptions of typical examples from the various crystal systems are given in condensed form in Table II. Practice in testing materials of known properties is the best means of acquiring skill in the determination of optical characteristics and the use of published data.

Outline for the Examination of Crystals.

Properties of the sample, as received.

Behavior on dissolving or heating.

Phenomena of crystallization. Influence of nature of solvent, temperature, etc. Allotropy, hydrates, double salt formation.

Geometrical form (habit). Faces, angles. Symmetry. Three-dimensional shape. Drawings of different views and habits. Directions of crystallographic axes. Miller indices of prominent faces. Crystal system.

Optical properties. With one nicol prism: Intensity of shading, and variation with change in the plane of vibration of polarized light. Refractive index of different components. Color, and pleochroism.

Between crossed nicols: Orthoscopic observation — Isotropic or anisotropic character. Positions of extinction, parallel or oblique. Extinction angles. Strength of double refraction, from polarization colors. Orientation of fast and slow components, for different views.

Conoscopic observation — Interference figure. Uniaxial or biaxial character. Sign of double refraction. Axial angle, $2V$ or $2E$. Plane of optic axes. Direction of acute bisectrix, Bx_a.

Optical orientation.

Refractive indices.

Sources and Use of Crystallographic Data. — The microscopist who is concerned with the recognition of a limited number of materials by optical tests may depend upon his own initial observations, as carried out on known specimens, for the determination of the necessary determinative characteristics. In dealing with a wide variety of substances, which may be more or less familiar to the observer, reliance must be placed on published descriptions for their identifications. It is always advisable to compare the specimen with a known sample of the material which it is thought to be, as a check on such procedure.

The various optical properties of all the common transparent minerals have been systematically tabulated, and are readily utilized for identification.[59] The chemist will find such mineralogical tables useful in many instances. Unfortunately, there is no such complete tabulation of the properties of the common chemical substances. Many relatively familiar compounds have never been described optically or crystallographically, and in using the tables which exist there is no assurance that the unknown material will be included in them. However, if there is the slightest clue as to the chemical nature of the material (such as may be gained from microscopic qualitative analytical tests) the published data are invaluable as a means of confirming tentative identifications or of deciding between a definite number of possibilities.

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[59] Larsen: Tables for the Identification of the Non-Opaque Minerals, *U. S. Geol. Survey. Bull.* **679** (1921).

Winchell: *Elements of Optical Mineralogy. Part II* (John Wiley & Sons, New York, 1927).

The monumental work of Groth[60] gives the crystallographic and optical properties of all substances described up to the date of publication, chemically similar materials being grouped together. References to the original publications are given in each instance, with drawings of ideal forms of most substances. The data for inorganic salts have been tabulated by Fry,[61] and Winchell has extended and rearranged this information in more detailed and useful form.[62] Crystallographic characteristics and optical orientations of organic substances have been tabulated by Keenan and Hann.[63] The simpler properties of a considerable number of medicinals and alkaloids are given by Mayrhofer,[64] and by Behrens and Kley.[65]

Published descriptions of individual substances sometimes include their crystallographic features, in enough detail to be useful for identification.

In making use of the descriptions of crystalline materials, it must be borne in mind that the forms shown are often ideal ones, rather than those which are likely to be obtained under ordinary conditions of rapid crystallization, on a microscope slide or even on a larger scale (see page 341). The habit of the crystals may be misleading if the observer is inexperienced in microscopic examinations. Furthermore, in the orthorhombic, monoclinic, and triclinic systems, the location of the crystallographic axes is to some extent arbitrary, so that the optical orientation with reference to them may sometimes present apparent discrepancies. Angular measurements, unless made between faces or edges which can be measured microscopically, are not of great value in rapid identifications without the use of a goniometer.

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[60] *Chemische Krystallographie*, 5 Vols. (W. Engelmann, Leipzig, 1906–1919).

[61] Tables for the Microscopic Identification of Inorganic Salts, *U. S. Dept. Agriculture, Bull.* **1108** (1922).

[62] *The Optic and Microscopic Characters of Artificial Minerals* (Univ. of Wisconsin Studies in Science, No. **4**, 1927).

[63] *International Critical Tables*, Vol. I (1926), pp. 320–338. (Only refractive indices and crystal systems are given for inorganic substances, in these Tables.)

[64] *Mikrochemie der Arzneimittel und Gifte* (Urban & Schwarzenberg, Berlin, 1928), 2 Vols.

[65] *Organische mikrochemische Analyse* (L. Voss, Leipzig, 1922).

Applications of Studies of the Optical Properties of Crystals. — Examinations of crystals with respect to the properties discussed in the preceding pages permit some ten or twelve different characteristics to be observed; about half of these are of a numerical character. Even with rather approximate determinations of the various qualitative and quantitative properties, the probability of another substance having an identical set of properties is roughly less than one in a million. If fewer properties are observed, the characterization is less exclusive, but even a brief examination with a simple polarizing microscope may supply more points of identification than an extended series of chemical tests. Since all this physical information is obtainable *in addition to whatever chemical information is at hand*, the positiveness of identification and description of crystallizable substances is increased enormously.[66]

Microscopic examination surpasses chemical study in the recognition of the actual solid phase present; by way of illustration the sodium orthophosphates may be taken. Their abbreviated descriptions are as follows:

$NaH_2PO_4 \cdot H_2O$. Orthorhombic; short prismatic crystals.
$\alpha = 1.456$. $\beta = 1.485$. $\gamma = 1.487$.
Double refraction, negative. $2E = 44°$.

$NaH_2PO_4 \cdot 2H_2O$. Orthorhombic; prismatic or pyramidal crystals.
$\alpha = 1.440$. $\beta = 1.463$. $\gamma = 1.481$.
Double refraction, negative. $2E = 150°$.

$Na_2HPO_4 \cdot 7H_2O$. Monoclinic; crystals thick basal plates.
$\alpha = 1.441$. $\beta = 1.442$. $\gamma = 1.453$.
Double refraction, positive. $2E = 57°$.

$Na_2HPO_4 \cdot 12H_2O$. Monoclinic; prismatic crystals.
$\alpha = 1.432$. $\beta = 1.436$. $\gamma = 1.437$.
Double refraction, negative. $2E = 86°$.

$Na_3PO_4 \cdot 10H_2O$. Cubic; octahedra, rhombic dodecahedra.
$n = 1.450$.
Isotropic.

$Na_3PO_4 \cdot 12H_2O$. Hexagonal; prisms.
$\epsilon = 1.453$. $\omega = 1.447$.
Double refraction, positive.

[66] Frey: *Science* **42**, 89 (1915).
Wright: *Jour. Amer. Chem. Soc.* **38**, 1647 (1916).
Chamot: *Ind. Eng. Chem.* **10**, 60 (1918).
Keenan: *Jour. Amer. Pharm. Ass.* **10**, 331 (1921); **14**, 112 (1925); **16**, 837 (1927).

Even on the basis of the few properties given, the differences are obvious, and it is evident that no very exact determinations are required to differentiate between these substances. The details of the form and optical orientation of the crystals serve further to emphasize the distinction between them, and the actual examination is the work of a few minutes as compared with the time required for a quantitative analysis.

Crystals may be identified in mixtures, and without removal from their mother liquor. Physico-chemical properties which might otherwise be overlooked are made apparent, and the homogeneity of samples for quantitative study is easily tested.

In the synthesis of a compound, its properties may be compared with crystallographic descriptions of the substance sought, and, since the possibilities are limited, identity or difference is quickly established; if an actual sample is available for comparison, such tests are even simpler. Even when the compound has never before been prepared or described, it may sometimes be recognized by comparison with material of analogous formula and properties, which is likely to be isomorphous with it (page 333).

Substances with complex formulas, which are not easily differentiated by chemical tests, are particularly appropriate subjects for such examinations, and their optical and crystallographic characteristics should form part of the general description of their properties.[67] The value of independent confirmation of chemical identifications is especially manifest in the field of organic chemistry, and in the examination of drugs and biochemical substances.

Isomers rarely crystallize with the same form and optical properties, and their differentiation is simple by direct examination; if the substances are liquid at ordinary temperatures, their crystalline derivatives may be compared.[68] The classic achievement of Pasteur[69] in recognizing the enantiomorphous forms of crystals of sodium ammonium tartrate may be mentioned as the prototype of all later investigations on optical antipodes. Such studies require well formed crystals of good size, as well as a considerable knowledge of crystallography.

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[67] As typical examples, such descriptions are given for a number of amino acids by Keenan: *Jour. Biol. Chem.* **62**, 163 (1924); the rare sugars are similarly tabulated: *Jour. Wash. Acad. Science* **16**, 433 (1926). See also Wherry: Application of Optical Methods of Identification to Alkaloids and Other Organic Compounds, *U. S. Dept. Agr. Bull.* **679** (1918); and *Jour. Amer. Chem. Soc.* **49**, 578 (1927).

[68] Chamot and Pratt: *Jour. Amer. Chem. Soc.* **31**, 922 (1909).
Hann and Keenan: *Jour. Phys. Chem.* **31**, 1082 (1927).
Migita: *Bull. Chem. Soc. Japan* **3**, 191 (1928).

[69] *Ann. de Chim. et Phys.* **24**, 442 (1848).

Allotropic modifications of crystalline materials invariably exhibit different crystal properties, and may thus be conclusively differentiated. The transformations of the various phases may also be studied by means of the polarizing microscope.[70]

One of the great advantages of crystallographic identifications, especially in biochemical work, is that a very small amount of material suffices for the study of form and optical properties. If necessary, even the few tiny crystals utilized may be recovered and analyzed chemically by micro-methods.

The above applications of crystallographic identifications are widely useful in chemical and allied industries. Single and double salts separating from concentrated liquors, intermediates, mixtures or adulterated materials, and in fact an infinite variety of solid substances may be positively and rapidly recognized. Samples may be studied while the material is "in process"; conditions of reactions may be controlled to give the maximum yield of desired isomers and the minimum of others; phase diagrams may be checked under working conditions; the nature of deteriorations may be ascertained.

The crystallographic microscope is widely employed in the mineral industries, for examinations of raw materials[71] and study of the changes which they undergo in the manufacturing operations. The various systems of silicates and aluminates such as are found in portland cement[72] and other ceramic products, the constitution of porcelain,[73] and of refractories,[74] the ingredients and constituents of glass,[75] are only a few of its many applications in the general field of ceramics.[76] Pigments[77] and fillers, natural and artificial abrasives, fertilizer ingredients,[78] soil-forming minerals,[79] natural deposits of salts,[80] and numerous other mineral materials have been studied and described by optical methods.

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[70] Wright: *Jour. Amer. Chem. Soc.* **39**, 1515 (1917).
Bowen: *Jour. Phys. Chem.* **30**, 721 (1926).
See also page 356.

[71] Arnold: *Jour. Amer. Ceram. Soc.* **6**, 409 (1923).

[72] Rankin: *Ind. Eng. Chem.* **7**, 466 (1915); *Jour. Franklin Inst.* **181**, 747 (1916).

[73] Klein: Constitution and Microstructure of Porcelain. *Bur. Standards Technol. Paper,* **80** (1916).

[74] Curtis: *Jour. Amer. Ceram. Soc.* **11**, 904–16 (1928).
Boericke: *Mining and Metallurgy* **10**, 16 (1929).

[75] Bowen: *Jour. Amer. Ceram. Soc.* **2**, 261 (1919).
Insley: *Idem.* **11**, 812–28 (1928); **12**, 143–52 (1929).

[76] Peck: *Jour. Amer. Ceram. Soc.* **2**, 695 (1919).

[77] Merwin: *Proc. A.S.T.M.* **17**, **II**, 494 (1917).

Opaque crystalline materials, such as minerals, may exhibit anisotropic character which can be determined by means of the polarizing microscope, using reflected light.[81]

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[78] Fry: Identification of Commercial Fertilizer Materials, *U. S. Dept. Agr. Bull.* **97** (1914).

[79] McCaughey and Fry: *U. S. Dept. Agr. Bur. Soils, Bull.* **91** (1913).

[80] Wetzel: *Caliche* **4**, 538 (1923); *Chem. Erde* **3**, 375 (1928).

[81] Schneiderhöhn: *Anleitung zur mikroskopische Bestimmung und Untersuchung von Erzen und Aufbereitungsprodukten im auffallenden Licht* (Berlin, 1922).

Sampson: *Econ. Geol.* **24**, 412–23 (1929).

Wright: *Proc. Amer. Phil. Soc.* **58**, 381 (1919).

TABLE II
OPTICAL PROPERTIES OF TYPICAL DOUBLY REFRACTIVE CRYSTALS

Name	System	Refractive Indices				Optic Axial Angle	Optical Orientation	Habit
		α or ω	γ or ϵ	β				
Nickel sulphate hexahydrate	T	1.511	1.487		−			Prism and bipyramid of same order, or tablets (001); lying on 001 or 110
Beryllium sulphate tetrahydrate	T	1.472	1.439		−			Pipyramids; lying on 111
Ammonium dihydrogen phosphate	T	1.525	1.479		−			Prism and bipyramid of same order; lying on prism face 110
Potassium dihydrogen phosphate	T	1.509	1.468		−			Same
Ammonium dihydrogen arsenate	T	1.577	1.522		−			Same
Potassium dihydrogen arsenate	T	1.567	1.518		−			Same
Ammonium copper chloride dihydrate	T	1.670	1.645		−			Prism and bipyramid of different order; lying on prism face 100
Potassium copper chloride dihydrate	T	1.637	1.615		−			Same
Mercuric cyanide	T	1.645	1.492		−			Same
Normal sodium orthophosphate dodecahydrate	H	1.446	1.452		+			Long thin primss; lying on prism face
Strontium chloride hexahydrate	H	1.536	1.487		−			Same
Strontium antimonyl tartrate	H	1.683	1.587		−			Prism and pyramid of same order; lying on prism face
Sodium nitrate	H	1.587	1.336		−			Rhombohedron; lying on rhombohedron face

TABLE II
OPTICAL PROPERTIES OF TYPICAL DOUBLY REFRACTIVE CRYSTALS. — *Continued*

Name	System	Refractive Indices				Optic Axial Angle	Optical Orientation	Habit
		α or ω	γ or ϵ	β				
Ammonium sulphate	O	1.521	1.536	1.523	+	$2V = 52°$	$Z = Bx_a = a$ Axial plane 010	Tablets, prisms; lying on 100 face
Potassium sulphate	O	1.494	1.497	1.495	+	$2V = 67°$	$Z = Bx_a = c$ Axial plane 100	Tablets, prisms; lying on 100 face
Potassium nitrate	O	1.335	1.506	1.506	−	$2V = 7°$ $\rho < v$	$X = Bx_a = c$ Axial plane 010	Tablets, prisms; lying on 010 face
Ammonium perchlorate	O	1.482	1.488	1.483	+	$2V = 69°$ $\rho > v$	$Z = Bx_a = b$ Axial plane 001	Tablets; prisms lying on 110
Silver nitrate	O	1.729	1.788	1.744	+	$2V = 62°$ $\rho < v$	$Z = Bx_a = c$ Axial plane 100	Tablets, lying on 001
Sodium sulphate decahydrate	M	1.394	1.398	1.396	−	$2E = 123°$	$Z \wedge c = 25–30°$. $X = b$ Axial plane $\perp$ 010	Prisms, elongated || b
Barium chloride dihydrate	M	1.635	1.660	1.646	+	$2V = 86°$ $\rho > v$	$Z \wedge c = 8°$ Axial plane 010	Tablets, flattened || 010 and bounded by {101}. $\beta = 89°$
Borax	M	1.447	1.472	1.470	−	$2V = 39°$	$Z \wedge c = 56°$ Axial plane $\perp$ 010	Tablets, flattened || 100; prisms, lying on 010
Sodium thiosulphate pentahydrate	M	1.489	1.536	1.508	+	$2V = 81°$ $\rho > v$	$X \wedge c = 41.5°$ Axial plane $\perp$ 010	Oblique ended prisms, lying on 010. $\beta = 76°$
Potassium chlorate	M	1.408	1.523	1.517	−	$2V = 27°$ $\rho > v$	$X \wedge c$ 48° Axial plane $\perp$ 010	Rhomb-shaped tablets, flattened || 001; opposite obtuse angles often truncated by $10\bar{1}$. $\beta = 80°$
Cupric sulphate pentahydrate	Tr	1.516	1.546	1.539	−	$2V = 56°$		Rhomb-shaped tablets, flattened || $\bar{1}10$
Boric acid	Tr	1.340	1.459	1.456	+	$2V = 7°$	X nearly $\perp$ 001 Axial plane nearly || b	Six-sided or oblique plates, flattened || 001. Prisms, elong. || b
Potassium bichromate	Tr	1.720	1.820	1.738	+	$2V = 52°$ $\rho > v$	Z nearly $\perp$ 010. Axial plane nearly $\perp$ 001	Prisms; tablets flattened || 010

CHAPTER X

CHEMICAL CRYSTALLOGRAPHY; PREPARATION OF CRYSTALS FOR STUDY

Crystallization is of such universal application in all fields of chemistry that a knowledge of the fundamental principles which govern it is indispensable to an intelligent comprehension of any process in which a solid phase is involved. The separation of substances from solvents or from the melt and the purification of these substances depend on crystal formation. Control of fineness of granular materials is to a considerable extent accomplished by regulation of the conditions of crystallization, as in the case of sugar, salt, soda, borax, certain pigments, etc. The actual shape and surface character of the grains is similarly governed. The physical properties of aggregates of crystals, such as metals, ceramic products, and cements of various kinds, are largely dependent on the nature and arrangement of the crystals which compose them. Crystallographic concepts are being employed in an ever-increasing variety of chemical problems, and have added much to our understanding of the nature of matter.

Only the general principles of crystallization can be briefly discussed and exemplified here.[1] The various experiments outlined will aid in the understanding of these principles and will give practice in the manipulations which are almost invariably a preliminary to the geometrical and optical study of the crystals after they have been obtained.[2]

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[1] For a more extensive and thorough discussion of the nature, growth, and chemical relationships of crystals, see

Fock-Pope: *Introduction to Chemical Crystallography* (Oxford, 1895).
Groth-Marshall: *Introduction to Chemical Crystallography* (John Wiley & Sons, New York, 1906).
Tutton: *Crystallography*, 2 Vols. (MacMillan & Co., London, 1922).
Crystalline Form and Chemical Constitution (1926).
Rinne-Stiles: *Crystals and the Fine Structure of Matter* (E. P. Dutton, New York, 1922).
Wyckoff: *Structure of Crystals* (Chemical Catalog Co., New York, 1924).
Tammann-Mehl: *States of Aggregation* (Van Nostrand, New York, 1925).
Joffé: *Physics of Crystals* (McGraw-Hill Book Co., New York, 1927).
Bragg: *Concerning the Nature of Things* (G. Bell & Sons, London, 1925).
Introduction to Crystal Analysis (Van Nostrand, New York, 1929).

FUNDAMENTAL CRYSTALLOGRAPHIC CONCEPTS, AND CONDITIONS AFFECTING CRYSTALLIZATION

The **space lattice**, in which the atoms of a crystalline substance are arranged, is the basis of its symmetry and of its class within one of the six crystal systems. The lattice constitutes the essential nature of any given solid phase, and primarily governs its internal properties such as refractive index, hardness, etc. The external properties of crystals involve also the surrounding medium, and the geometrical faces exhibited are subject to variation even though the space lattice is wholly unaffected. Whatever may be the planes of atoms which constitute the surfaces of the crystal, the dimensions of the lattice remain constant, and the angles between corresponding faces do not vary whatever the relative areas of these faces. A face moved parallel to itself is unaffected, crystallographically.

The number of possible space lattices is limited, and more than one substance may crystallize with essentially the same arrangement of atoms, except for variations in dimensions. If two substances crystallize with similar space lattices, the dimensions of which are nearly identical, and if their chemical constitutions are analogous, the atoms of each kind are more or less mutually interchangeable without marked alteration of the structure. Such substances are said to show **isomorphism** or **solid solution**, and if several exist they constitute an **isomorphous series.**[3] Perfect replaceability throughout the entire range of compositions is rarely possible, but limited isomorphism is very common in the various " families " of elements and their compounds. Formulas for such isomorphous mixtures or " solid solutions " are commonly written thus: $(Rb,Cs)_2SO_4$, indicating that the elements in parenthesis may vary in relative amount without affecting the essential structure of the compound.

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[2] Some of these experiments are original; others have been suggested by those given in the following-named works, to which the reader is referred for further examples:

- Lehmann: *Molekular Physik* (Engelmann, Leipzig, 1888–89); *Die Krystallanalyse* (*idem*, 1891).
- Barker: *The Study of Crystals in Schools* (Oxford, 1920); *Practical Suggestions Towards the Study of Crystals in Schools* (Oxford, 1921).
- Groth: *Elemente der physikalischen und chemischen Krystallographie* (R. Oldenbourg, Munich, 1921).
- Vorländer: *Chemische Krystallographie der Flüssigkeiten* (Akademische Verlag, Leipzig, 1924).

[3] Tutton: *Crystallography*, Vol. II, Chap. 54.
Groth-Marshall: *Introduction to Chemical Crystallography*.
Bruni: *Chemical Reviews* **1**, 345–75 (1925).

As typical examples of isomorphous series may be cited the alums (Na, K, Rb, Cs, Tl, Ag, NH_4) (Al, Cr, Fe, Mn, In, Ga, Tl) $[(S, Se)O_4]_2 \cdot 12H_2O$; the alkali sulphates and selenates (K, Rb, Cs, Tl, Ag, $NH_4)_2$ (S, Se, Cr, Mn)O_4; the double salts (K, Rb, Cs, Tl, $NH_4)_2$ (Mg, Zn, Mg, Fe, Ni, Co, Cu, Mg) (S, Se)$O_4 \cdot 6H_2O$; the alkali arsenates, phosphates, and vanadates; strontium, barium and lead nitrates; perchlorates and permanganates; alkali chloroplatinates; and other less extensive series.

The dimensions of the space lattices of the various separate compounds which form an isomorphous series are not precisely identical, and the angles between corresponding crystal faces show slight differences (usually less than 2°). Ordinarily these dimensional discrepancies do not interfere with the mutual replaceability (at least to a limited extent) of like atoms, and the composite space lattice possesses dimensions of intermediate magnitude. Optical and other physical properties show a similar but usually more striking progressive change with composition. In certain cases actual replacement appears to be unobtainable, but the similarity of form and structure is such that the compounds are still considered isomorphous.

Isomorphous substances are capable of " seeding " supersaturated solutions of each other, and of being deposited in a layer as " overgrowths " on the surface of growing crystals of other members of the series.

Experiment 1. (**a**) — Crystallize ammonium perchlorate from solution, and observe its crystal form. Repeat, having enough potassium permanganate in the solution to color it pink. Note that the colored crystals possess the same form as the colorless ones.

(**b**) — Precipitate silver sulphate from dilute solution, and examine. Recrystallize silver chromate from dilute ammoniacal solution, and note the pleochroism of the crystals (page 286). Next precipitate silver sulphate in the presence of a *trace* of chromate, and note that its crystals are tinted and possess pleochroism.

(**c**) — Compare the crystal properties of cupric sulphate pentahydrate and ferrous sulphate heptahydrate. Then crystallize a solution containing both salts, and note that the form of the latter determines that of the crystals of $(Cu,Fe)SO_4 \cdot 7H_2O$.

(d) — Prepare a drop of a slightly supersaturated solution of ammonium chrome alum and " seed " it with a tiny crystal of potassium aluminum alum. " Overgrowths " may be obtained.

Isomorphism is of great help to the chemist; analogous substances may be grouped together and their probable behavior as solids correlated. Identifications by comparison with known material which is likely to be isomorphous with the unknown frequently afford a valuable check on the analyses of newly synthesized compounds. Valence relationships and formulas may be confirmed even when the materials are not obviously related chemically. The possible occurrence of impurities, the separation of mixtures by recrystallization, and the behavior of rare elements in analysis may be predicted. In fact, the entire field of our knowledge of solids may be greatly systematized by the application of this fundamental principal of crystallography.

The **degree of supersaturation or supercooling** of the phase from which crystals are to be formed is an important factor governing their size and number. In general, if this phase is rendered markedly metastable, a relatively large quantity of new crystalline material must be formed in order to relieve this condition. If, however, the saturation, freezing, or transition point is barely passed, crystal separation will be gradual and will be regulated by the rate of evaporation or heat transfer, provided the very slight differential is maintained.

The **number of crystal nuclei** formed determines the size of the crystals, since the amount of material separating is divided among them. The greater the degree of metastability, the more crystals will be started. Ideally, for the growth of good-sized crystals, the number of nuclei should be kept low, so that any crystallization will be about a few centers which will thus develop to a larger size. If a solution is supersaturated and crystallization is initiated by stirring or "seeding," a large number of very small crystals are formed. Local supersaturation is frequent, even while crystals are separating in adjacent portions of the same drop of solution.

Experiment 2. — Concentrate a drop of barium nitrate solution to supersaturation on a microscope slide. Scratch the surface of the slide with a glass rod, or seed with a tiny fragment of the solid, and note the size and number of crystals formed. Compare with those obtained by crystallizing while the drop is stirred or kept seeded.

The **rate of crystal growth** is also affected by the degree of metastability of the phase from which the crystals separate.[4] In general, the greater the supercooling or supersaturation, the faster the size of the crystals increases when once they start. However, this is counteracted by the increased resistance to the rearrangement of the atoms in the new space lattice. As a consequence extreme metastability tends to be maintained indefinitely, as in the case of glass or clear sugar candy drops.

Too rapid evaporation of a solution of a hydrated salt may supersaturate it beyond the range of rapid crystallization before any nuclei are formed, and a less hydrated form may appear. Allotropic transformations are frequently " suspended " by too rapid cooling, and can be made to proceed only at temperatures near the transition point (see page 357).

Experiment 3. (**a**) — Evaporate a drop of a solution of alum or cane sugar rather rapidly, without boiling or stirring, and note that a vitreous, highly concentrated " solution " finally results. Attempt to induce crystallization by stirring or seeding. Then add a very small quantity of water and again endeavor to start crystal growth.

(**b**) — Melt completely a crystal of thymol or *m*-bromnitrobenzene, beneath a cover-glass on a slide. Cool rapidly, and note that crystallization is delayed indefinitely, unless the melt is scratched or seeded.

(**c**) — Concentrate a solution of sodium tetraborate decahydrate (borax) without stirring, and note the formation of a supersaturated solution of high viscosity. If the solution is stirred after a moderate degree of supersaturation is reached, crystals of sodium tetraborate pentahydrate (" octahedral borax ") may be formed instead of those of the decahydrate.

(**d**) — Melt sulphur on a slide, beneath a cover-glass, and allow the monoclinic allotropic modification to crystallize. Note that it is apparently stable at room temperature, but that the transformation to the orthorhombic modification occurs fairly rapidly on careful warming.

If supersaturation or supercooling is marked, the crystals formed may be so minute as to exhibit no apparent structure, and the material is sometimes called " cryptocrystalline." However, the particles possess space lattices even when the grain size is very small, and "insoluble" substances are rarely precipitated in a truly amorphous state, even though of submicroscopic fineness.[5]

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[4] Freundlich: *Colloid and Capillary Chemistry* (1922), pp. 318–41.
Tamman: *op. cit.*, Chap. IX.
Findlay: *The Phase Rule* (London, 1927), p. 41.
Miers: *Jour. Inst. Metals* **37**, 331 (1927).

Experiment 4. — Examine under the microscope a number of the "amorphous" precipitates of analytical chemistry, such as silver chloride, barium sulphate, calcium carbonate, etc., and note that they are actually composed of exceedingly minute crystals.

The **habit** of a crystalline substance is markedly affected by external conditions, being primarily determined by the relative rates of deposition of atoms on different planes of the space lattice. The slower the deposition parallel to a given plane the larger the crystal face produced, and the more rapid the deposition the more that face tends to be built up toward a point.[6]

The rate of deposition of matter on the different planes of the space lattice of a growing crystal is influenced by the rate of crystallization. In general, with rapid growth only the more prominent faces are developed, whereas if crystal formation is slow many other "forms" may appear, as smaller faces truncating the angles between the larger faces. The "ideal" crystals commonly pictured have usually been obtained under conditions of very slow and uniform growth. Crystals developing under metastable conditions tend to grow fastest at their angles, thus maintaining relatively few faces and simple habit. However, if the rate of growth is too great, deposition of material at the edges and corners of the crystal will exceed that on its plane faces, and "hopper-shaped," dendritic, or skeletal habit will be produced. In the re-entrant angles thus formed, inclusions of the mother liquor may be entrapped.

Experiment 5. (**a**) — Precipitate potassium perchlorate by allowing drops of moderately concentrated solutions of potassium chloride and perchloric acid to run together on a slide. Note the dendritic character of the crystals, and compare with their normal habit as formed from very dilute solutions. Study similarly thallous chloride, magnesium ammonium phosphate.

(**b**) — Recrystallize sodium chloride rapidly from solution, and note the hopper-shaped crystals and zones of inclusions. Perform similar rapid crystallizations of mercuric chloride, urea, ammonium sulphate, potassium antimonyl tartrate.

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[5] Von Weimarn and Hagiwara: *Kolloid Zeits.* **38**, 129–36 (1926).

[6] Wherry: *Amer. Mineral.* **9**, 45 (1924).

Evans: *Chem. and Ind.* **44**, 791, 812 (1925).

Spangenberg: *Zeits. angew. Chem.* **39**, 304 (1926).

Desch: *Nature* **117**, 694 (1926).

Bentivoglio: *Proc. Roy. Soc. Lond.* **115A**, 59–87 (1927).

Saylor: *Jour. Phys. Chem.* **32**, 1441–60 (1928) gives an excellent series of figures illustrating this relationship.

The surface on which growing crystals lie restricts the deposition of material on one face, so that the bottoms of the crystals are usually flattened. This distortion may be avoided if crystals can be grown suspended in liquid, as in the case of materials precipitated by metathetical reaction between two solutions. Frequent stirring is necessary if crystallization is carried out in a drop on a microscope slide, in order that the crystals may be turned about and that supersaturation may be avoided. If the drop of liquid is too shallow, the growth of crystals upward is also restricted, so that they are further flattened. In correlating different views of the same substance, exhibited by crystals lying on different faces, allowance must be made for this tendency to flatten parallel to the slide (Fig. 79).

The variation in habit exhibited by a single substance under substantially constant conditions of crystallization is often very extensive.[7]

Twinning sometimes occurs in crystals prepared for microscopic examination. It is sometimes evident as a union of two or more well formed crystals, but may also occur within a geometrical boundary like that of a single crystal. Twin crystals have one plane of atoms in common, but otherwise possess differently orientated space lattices. This difference of orientation is recognized in anisotropic materials by different positions of extinction for each portion of the twinned crystal. Barium chloride dihydrate often affords good examples of such internal twinning.

Adsorption of the solvent, or of material dissolved in it, preferentially upon certain atomic planes tends to retard deposition on these planes and they are manifest as crystal faces. In this way the crystal habit of a given substance may be markedly varied, without any alteration of its internal structure or composition.[8]

Experiment 6. (a) — Recrystallize sodium chloride from an aqueous solution of urea, and compare its habit with that from pure water. Perform similar experiments with potassium alum and urea; with barium nitrate and nitric acid.

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[7] As for instance, in the infinite variety of forms of snow flakes. An interesting series of photographs of the different types of crystals of silver bromide is given by Trivelli and Sheppard: *The Silver Bromide Grain of Photographic Emulsions* (Eastman Kodak Co., 1921).

[8] Saylor: *loc. cit.*

(b) — Recrystallize barium or lead nitrate from a concentrated aqueous solution of methylene blue and note that the crystals obtained are colored by the adsorbed dyestuff. Frequently the adsorption is noticeably greater on the cube faces, which are developed by this procedure.[9]

Colloidal material in solution may affect crystal habit very markedly, and in some cases may completely prevent normal development. The dendritic character of relatively soluble substances is greatly increased, whereas in the case of slightly soluble materials precipitation or recrystallization in the presence of a colloid ordinarily gives a much larger number of nuclei, and hence smaller and more uniform crystals.[10]

Experiment 7. — Recrystallize a number of salts which normally form well developed crystals from water, such as copper sulphate, ammonium sulphate, sodium chloride, mercuric chloride. Repeat the crystallization, using a warm concentrated solution of gelatin or gum arabic instead of pure water, and note the anomalous habit of the crystals which separate on standing.

See also Experiment 13 (d), page 347.

Certain substances have anomalous habit even under ordinary conditions of crystallization, and are rarely obtainable in definite forms bounded by plane faces. Their appearance may nevertheless be distinctive and useful for purposes of identification.

Dendrites, consisting of branching crystals or skeletal aggregates, in tree-like forms, are commonly encountered, especially if crystallization is rapid or normal growth is interfered with. **Spherulites** of small crystals radiating from a common center, are more or less characteristic of certain substances. Their behavior between crossed nicols is particularly striking, since those crystals of the aggregate which lie in the positions of extinction appear dark and quarter the spherulite by a black cross. As the stage is rotated, other crystals move into the positions of extinction and hence the arms of the cross remain stationary; this often gives the illusion of their rotating within the spherulite, in a direction opposite to that of the stage. Curved growths are not uncommon, especially in minerals,[11] or certain organic compounds such as fats and waxes.

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[9] Gaubert: *Recherches récentes sur les facies des cristaux* (Paris, 1911); *Comptes rendus* **157**, 1446, 1531 (1912); **159**, 486 (1914); *Rev. gén. sci.* **37**, 357–66 (1926).

[10] Ord: *Influence of Colloids upon Crystalline Form and Cohesion* (London, 1879).

Alexander: *Kolloid Zeits.* **4**, 86 (1909).

Lowndes: *Trans. Faraday Soc.* **16** (1920), Appendix, p. 128.

Anderegg and Daubenspeck: *Proc. Indiana Acad. Sci.* **34**, 171 (1925).

Eckert and France: *Jour. Phys. Chem.* **31**, 877 (1927).

Experiment 8. (a) — Recrystallize ammonium chloride from water, and note the almost unavoidable formation of dendrites.

(b) — Add a tiny fragment of metallic zinc to a drop of a solution of one of the following: lead nitrate, silver nitrate, copper sulphate. Observe the tree-like or mossy crystals which form.

(c) — Evaporate rapidly a film of an alcoholic solution of *m*-nitrobenzoic acid, and study the tiny spherulites between crossed nicols.

(d) — Recrystallize the following from fusion in a thin layer beneath a cover-glass, and examine the spherulites between crossed nicols: cinchonidine, *o*-nitrophenol (pleochroic; study also with one nicol); oleomargarine.

(e) — Mount potato or arrowroot starch in water, and examine the grains between crossed nicols.

(f) — To a drop of a solution of barium chloride add a tiny fragment of sodium acetate. Then precipitate barium oxalate by the addition of a solution of oxalic acid, and note the sheaves and fibrous needles which separate.

Repeat the experiment, having barium chloride, sodium acetate, and ferric chloride in the drop to which the oxalic acid solution is added. Observe the fine hair-like flexible crystals which form

Pseudomorphic habit occurs when a secondary phase crystallizes so as to occupy the same space as did the primary crystal. Ordinarily such growth does not result in well formed crystals, but rather in aggregates which lie within the boundary of the original crystal. Pseudomorphs rarely have such well formed outlines or homogeneous structure and optical properties as to obscure the fact that their external shape is unrelated to their final composite crystalline nature. Effloresced salts and allotropic modifications formed from the solid state afford the most common examples of pseudomorphism in microscopic studies.

The phenomenon of **rhythmic crystallization** (Liesegang's rings)[12] may be demonstrated microscopically.

Experiment 10. — Dissolve in a large drop of 10 per cent gelatine solution a little potassium arsenate. Spread the drop on a slide to form a layer about 1 mm. thick, and cool until the gelatin has set. Then place at the center of the layer a tiny drop of silver nitrate solution, acidified with nitric acid. Several trials may be necessary, in order to secure exactly the right conditions, but concentric rings of fine-grained precipitated silver arsenate will be obtained. Similar phenomena may be obtained with potassium bichromate and silver nitrate.

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[11] Spencer: *Mineral. Mag.* **19**, 263 (1921).

[12] Dhar and Chatterji: *Kolloid Zeits.* **37**, 2, 89 (1925).
Von Weimarn: *ibid.* **37**, 78 (1925).
Alexander: *Colloid Chemistry,* **I** (1926), pp. 782, 790, 796.
Hedges and Myers: *Physico-Chemical Periodicity* (London, 1926), Chap. III.

METHODS OF OBTAINING CRYSTALS FOR MICROSCOPIC STUDY

One of the great advantages of the use of the microscope in the examination of crystals lies in the fact that the specimens are very easily prepared, as compared with the elaborate methods required if other instruments are employed. Small crystals suffice, and these need not be highly perfect, since a number may be studied and the observations combined. The saving of time in growing crystals for microscopic observation, as compared with that required for macroscopic preparations, measurements, and tests, is enormous, and the results, though less exact, are accurate enough for purposes of identification and ordinary comparison.

Crystallization on a macroscopic scale is frequently a more convenient means of obtaining suitable specimens than are microcrystallization procedures. The rate of evaporation, cooling, or mixing of the phase from which the crystals form is more accurately controllable in beakers, crystallizing dishes, or plant apparatus than on a slide under the microscope. Concentrations may be more exactly regulated; pressure, vacuum, or an inert atmosphere may be employed; the large volumes of liquid necessary for the crystallization of slightly soluble materials are easily handled; and a valuable instrument is not monopolized during prolonged operations. In many instances the extremes of temperature required preclude carrying out the crystallization under the microscope. Occasionally the crystallization process is so closely related to a chain of chemical operations that it necessarily must take place under exacting conditions; this is particularly true of certain syntheses, especially those conducted on a plant scale.

The ultimate crystalline product of operations which are carried out with grams or tons of material is as easily examined microscopically as if a few milligrams were prepared on a slide, even if its actual formation was not followed. A small amount of such material may be collected, at various stages in the process if desired, and it can be mounted in an appropriate liquid, or in its mother liquor, for microscopic study. Samples prepared under different conditions can be compared, and detailed observations of size, form, optical properties, and other characteristics may be made just as already described. The chief precaution which needs to be observed is the avoidance of any change in the state of aggregation or physico-chemical nature of the material during its preparation for study; knowledge of the chemistry of the system should obviate such possibilities.

The metallographic study of alloys depends chiefly upon the observation of the properties of material which has been crystallized on a large scale, the final product being utilized for microscopic examination of the size, shape, and arrangement of its crystals.

Crystallization on a microscopic scale has the great advantage that crystal growth can be followed directly, and physico-chemical changes which might otherwise be missed can be noted as they occur. In addition, the operations are exceedingly rapid, and a whole series of observations may be carried out in a short time. The lack of perfect uniformity in conditions throughout the slide often permits a considerable range of temperatures, rates, or compositions to be studied in a single preparation.

Whenever possible, both macroscopic and microscopic crystallization procedures should be employed, as complementary to each other. Growth phenomena are best studied under the microscope; growth conditions, on a larger scale. Crystals grown under the microscope are likely to be smaller, but more perfect, than those prepared in greater quantities, unless exact control of conditions is maintained. Frequently the method of crystallization carried out on a microscope slide may be adapted from that used in a manufacturing operation in the plant, or in macroscopic laboratory apparatus, with an essential duplication of the phenomena and the opportunity of observing the various stages while they are taking place. The various procedures employed in the formation of crystals on a micro scale exemplify many important physico-chemical principles, and are constantly employed in microscopic analyses.[13]

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[13] Examples and applications of a number of different types of crystallization methods are discussed by Denigès: Klein and Strebinger's *Fortschritte der Mikrochemie* (Leipzig, 1928), pp. 21–33.

Micro-crystallization from solution is the most commonly employed means of obtaining crystals for microscopic study. Water is used as a solvent when possible, but acids, alkalies, or organic liquids may also be utilized. Frequently the solubilities and habit vary so widely in different solvents that crystallization from more than one is necessary for full information.

In general the **method of crystallization on a microscope slide** is as follows: Place a drop of water at the corner of a clean object slide (preferably half-size); the drop should be 5 to 7 mm. in diameter, and 1 to 2 mm. deep. If larger than this, tilting the slide will cause it to run; if smaller, its surface is likely to be so rounded as to interfere with observation of crystals within it. Introduce into the drop a tiny fragment of the substance to be recrystallized; the quantity used should be just enough to saturate the drop, and it should be added in small portions no larger than this o, with stirring each time. Large particles may be crushed by the tip of the glass rod (Fig. 69) or spatula (Fig. 70) used for handling the material. In stirring, the rod should be held nearly upright, in order to avoid spreading the drop to form a thin film on the slide. Solution may be hastened by cautiously warming the preparation over the micro-burner, but care should be taken to avoid rapid evaporation. A crust of imperfect crystals will form around the edges of the drop, in the course of a few seconds, especially if the solution is allowed to cool, or if evaporation is accelerated by blowing across it. This crust is of no value for study and should be pushed into the center of the drop, which may still be unsaturated; in doing so the rod should be held vertically, and spreading the drop or scratching the slide should be avoided. Evaporation should be continued, preferably without further heating, the solution being kept "seeded" by gently stirring in the crystals which separate at its edge. As soon as crystal growth is well under way, the slide should be cooled, by pressing it against a cold object, so that evaporation will proceed slowly at room temperature. The examination of the crystals may now be begun, but occasional stirring is necessary to prevent localized supersaturation in the drop as it evaporates further.

As soon as evaporation has gone so far as to leave the growing crystals projecting above the surface of the drop, their faces become imperfect, and recrystallization is again necessary. This is accomplished by placing a *very* tiny drop of water on the mass of crystals, by means of the stirring rod; a dropping pipette should not be used for this purpose. Rather less than enough liquid to redissolve the crystals should be added; in this way some of them remain to " seed " the next crop, and no time is lost in evaporating excess solvent before regaining the saturation point. The crystallization procedure is continued as above. Several recrystallizations of a single preparation are ordinarily possible without loss of material, and are usually necessary in order that the crystals may always be studied while completely immersed in their mother liquor and before they have grown into contact with each other.

The manipulative technique as outlined above is subject to modification, depending on the nature of the solvent and solute. " Insoluble " substances should be added to the drop in very small amount, since the excess will not dissolve and may interfere with the observation of the portion which does recrystallize. Warming the solvent, with a minimum of evaporation, will facilitate solution. Only very small crystals will be formed at best, and to obtain them the solution should be evaporated or cooled very slowly.

Highly soluble substances will ordinarily crystallize very rapidly when once the solution is saturated, for a very little further evaporation results in the separation of a large amount of material, often in dense masses unsuitable for study. For this reason supersaturation should be guarded against, and as soon as the saturation point is reached the solution should be thoroughly cooled, and stirred well to break up aggregates of crystals. A cover-glass may be placed over the drop, to retard evaporation, after crystallization has begun. In recrystallizing highly soluble materials the minimum quantity of solvent should be added; often simply breathing on the crystals is sufficient.

Substances much more soluble in hot water than in cold may best be crystallized by cooling, rather than by evaporation. However, it is usually unnecessary to saturate the hot solution in order to have crystals separate on cooling; moderate concentrations are ordinarily sufficient to give a good crop of crystals, not too densely distributed for study.

Supersaturation is sometimes very troublesome, and although gentle stirring is ordinarily sufficient to relieve it, " seeding " with a fragment of fresh material may sometimes be necessary.

The rate at which such a seed crystal dissolves is an indication of whether the solution is saturated or not, and particular care should be taken not to continue rapid evaporation after saturation is reached, or a vitreous, non-crystallizable film will result.[14]

Deliquescent materials are particularly difficult to crystallize if the atmosphere is humid. A drop of solution may be placed in an ordinary dessicator, and as soon as crystallization has begun may be covered with a cover-glass and placed under the microscope for study. A better procedure is to crystallize in a hanging drop on the lower surface of a cover-glass which is placed over a hollow slide containing a dessicant such as phosphorus pentoxide (Fig. 123). The edges of the cavity may be sealed with vaseline if desired. Recrystallization is accomplished by exposing to the air for a moment, and replacing over the micro-dessicator.

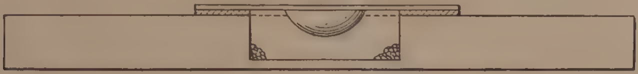

FIG. 123. Crystallization of a Deliquescent Material in a Hanging Drop over a Dessicant.

Hydrolysis of certain metallic salts is likely to occur if it is necessary to heat the substance for some time in water, or if repeated recrystallizations have been made. It may best be prevented by a trace of the appropriate acid.

Double salts when recrystallized may form one of the single salts before the proper molecular ratio of concentrations in the solution is reached. Further evaporation will give rise to a second phase, if this is the case. Mixtures of salts may interact as they are dissolved, and should be watched as they are introduced into the solvent.

Organic solvents may be handled like water, provided they are not too volatile or too mobile. Where feasible, the less volatile of the possible solvents should be chosen; as for instance, xylene instead of benzene. Crystallization from very volatile solvents may be controlled by covering with a cover-glass to retard evaporation. Such solvents and other organic liquids of low surface tension, tend to creep over the slide and are difficult to maintain in a sufficiently thick layer for proper crystal growth. To aid in their manipulation tiny watch glasses or concave-ground slides may be used. Crystallization may also be carried out in small glass crucibles (Fig. 62, *C*), which are particularly useful for volatile solvents; the crystals can be examined without removal. If the crystallization has been made in a test tube, crystallizing dish, or other vessel, the crystals may be pipetted to a microscope slide for study.

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[14] The importance of supersaturation, and the methods of preventing it, are discussed by Denigès: *Mikrochemie* **3**, 33 (1925), who suggests rubbing the slide with a crystal of the substance before placing the supersaturated drop on the same place.

Howard and Stephenson: *U. S. Dept. Agr. Bur. Chem. Bull.* **137**, p. 189, state that stirring rods previously used in the crystallization of a substance may carry sufficient traces of it to induce crystallization in subsequent preparations.

Experiment 11. (**a**) — Recrystallize the following from alcohol, by evaporation: thymol, sulphonal, or salol.

(**b**) — Recrystallize the following from xylene, by evaporation: iodoform, naphthalene, thymol, or salol.

(**c**) — Recrystallize the following, by dissolving in the warmed solvent and cooling: stearic acid, from cottonseed oil; paraffin wax, from kerosene (examine between crossed nicols, using " 1st order red " plate); sulphur, from aniline (two allotropic forms may be observed).

Variations in the composition of the solvent afford means of crystallizing inorganic or organic substances. Volatile acids or ammonia may be used to aid solution, and allowed to escape on standing, with consequent gradual separation of the dissolved material. The familiar " salting out " procedure may be applied to micro-crystallization. Acids or alkalies may be neutralized by slow diffusion into the drop, with reprecipitation of the substance which they held in solution. Alcohol may be used as a solvent, and diluted with water to cause crystallization of the dissolved material.

Experiment 12. (**a**) — Dissolve silver chloride in ammonium hydroxide, and allow to stand in the air. Note the separation of crystals as ammonia escapes.

(**b**) — " Salt out " sodium benzene sulphonate from a drop of its aqueous solution, by the addition of sodium chloride, solid or in saturated solution.

Precipitation from solution as a result of chemical reaction is an excellent means of preparing crystals of many relatively insoluble materials. Most of the metathetical reactions which yield an insoluble product may be duplicated on a microscope slide, and the precipitates studied; this is the general procedure employed in microscopic qualitative analysis. The various methods of bringing the reacting substances together are discussed in detail in Volume II.

The factors governing crystallization are applicable to precipitation methods, and serve to control the size and perfection of the crystals produced. Low concentrations of the reacting substances are necessary, if good crystals are to be obtained; this is particularly true of highly insoluble materials, which can be precipitated in recognizable form only from exceedingly dilute solutions. The rate of mixing of the interacting compounds is closely related to their effective concentrations, and should be very slow and gradual.[15] The temperature affects the size of the crystals precipitated, by governing their solubility somewhat, and also because they " digest " more rapidly to form larger crystals when warmed. Increasing the solvent action of the liquid, as when precipitating in the presence of certain acids or alkalies, also tends to give a coarser precipitate. Protective colloids, on the other hand, tend to render the precipitate very fine grained. A large excess of one of the reagents may result in the formation of a double salt or other soluble compound, and prevent precipitation or dissolve the crystals after they have formed.

Experiment 13. — Prepare large drops of moderately dilute solutions of lead nitrate and potassium iodide. Use these drops as stock solutions, to furnish the same concentrations throughout the following series of *comparative* experiments:

(a) — Allow a drop of a solution of potassium iodide to flow into one of lead nitrate, on an object slide, and note the size and character of the crystals obtained. Repeat, using solutions about twenty times as dilute, and compare the precipitate with that obtained from the first reaction. (*Cf.* Experiment 5, page 337).

(b) — Precipitate by mixing hot solutions, and compare the crystals with those obtained in the cold.

(c) — Acidify a drop of the lead nitrate solution with nitric acid, and precipitate with potassium iodide, comparing the crystals with those from neutral solution.

(d) — Dissolve a small amount of gelatine in warm water, and dilute each of the solutions with it. Mix, and observe the character of the precipitate.

(e) — Add an excess of solid potassium iodide to a drop of the lead nitrate solution. Observe whether precipitation occurs, and note the separation of a double salt $PbI_2 \cdot KI \cdot 2H_2O$ on evaporation.

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[15] Very remarkably large crystals of slightly soluble materials have been obtained by reactions between solutions diffusing slowly into gels. Holmes: *Jour. Amer. Chem. Soc.* **40**, 1187 (1918); *Jour. Phys. Chem.* **21**, 709 (1917). See also Martini: *Mikrochemie* **7**, 236 (1929).

Experiment 14. — Add a drop of an ammoniacal solution of magnesium chloride and ammonium chloride to a drop of a dilute solution of a phosphate. Compare the freshly precipitated ammonium magnesium phosphate hexahydrate with that obtained by allowing the precipitate to "digest" in its mother liquor at room temperature. Follow the changes which take place under the microscope.

Slightly soluble organic acids or bases may be dissolved in alkaline or acid solutions, and reprecipitated by the gradual addition of acid or alkali. If the solutions used are very dilute, such procedure affords a convenient means of recrystallizing materials which are otherwise difficult to handle in aqueous solution.

Experiment 15. (**a**) — Precipitate the free base from a dilute solution of quinine in dilute sulphuric acid.

(**b**) — Recrystallize the following by dissolving in dilute sodium hydroxide solution and acidification with hydrochloric acid: benzoic acid, salicylic acid, aspirin, or saccharin.

Occasionally substances with low melting points may be precipitated as droplets of supercooled liquid, which do not crystallize immediately.

Micro-crystallization from Vapor: Sublimation. — Sublimation is particularly valuable as a method of obtaining crystals of volatile substances which are very slightly soluble or otherwise difficult to crystallize. It also serves as a means of separating the constituents of mixtures, the non-volatile portions being left behind; a small amount of a sublimable ingredient may thus be collected from a large mass of other materials, as in testing vegetable drugs. The greatest application of sublimation is in the investigation of organic substances, though it is also useful in connection with certain inorganic compounds such as ammonium chloride, arsenic trioxide, mercuric iodide and chloride, etc.[16]

A number of different methods are available for micro-sublimation,[17] of which only the simplest will be described here.

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[16] Behrens-Kley: *Mikrochemische Analyse* (Leipzig, 1921), pp. 188, 195.

[17] Tunmann: *Pflanzenmikrochemie* (Berlin, 1913), pp. 23–32.

Houben-Weyl: *Die Methoden der Organischen Chemie* **1**, 630–43 (1921).

Molisch: *Mikrochemie der Pflanze* (Jena, 1921), p. 28.

Mayrhofer: *Mikrochemie der Arzneimittel und Gifte* (Berlin, 1923–28), I Teil, pp. 16, 263; II Teil, p. 11.

Emich: *Mikrochemisches Praktikum* (Munich, 1924), p. 40; *Lehrbuch der Mikrochemie* (Munich, 1926), p. 56; *Mikrochemie* **3**, 25 (1925).

If exact temperature control is not essential, the usual method is that of sublimation from one slide to another. The material to be tested is placed at the corner of a thin slide. If it is solid it is wise to moisten it with water and then dry it thoroughly; generally this will effectually prevent the material from being blown off by air currents, and brings the substance in intimate contact with the glass slide — a matter of prime importance. If the material is already in solution evaporate a tiny drop, but in this case it should not be spread out, as is commonly done with test drops. When the drop is dry, add another tiny drop on top of the residue left by the first; this in turn is dried, the process being repeated until, in the judgment of the operator, there is sufficient material for work. In all cases the residue to be treated should occupy but little space, yet should not be too thick, since, if fractional sublimation is to be practiced, a thick mass is apt to be heated unequally and fallacious results will be obtained.

Everything being ready, the slide is held in the left hand and the heating begun over the micro-flame, not directly beneath the spot of material, but slightly nearer the center of the slide. This is done in order to avoid raising the temperature too rapidly and too high. As soon as the sublimation point is almost reached (which can easily be recognized by practice) a second clean slide, carrying a drop or two of water, is taken in the right hand and lowered over the first slip, with the drop of water on the upper side directly over the material to be sublimed. The drop of water has for its object the keeping of the upper slide cool, thus more effectually condensing any vapors produced by the heating. The receiving slide is supported on an edge of the other and is brought as close to the substance as is possible without touching it (Fig. 124). The temperature is gradually raised by moving the spot of substance nearer the flame. As soon as there is evidence of the appearance of a sublimate, raise the two slides

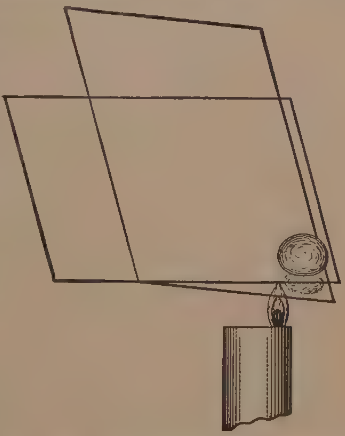

Fig. 124. Sublimation of Material from One Object Slide to Another.

above the flame so as to prevent too rapid vaporization. The first deposit being obtained, the receiving slide is moved along a few millimeters and a second sublimation made; again the slides are partly removed from the source of heat, the receiving slide moved along a trifle, and again the temperature is raised until a third film has been condensed. The process is continued as long as the material remains on the first slide or fails to yield any further sublimate. If the drops of water, used to keep the receiver cool, evaporate, replace them by others. When dealing with compounds which melt on heating, the supporting slide must be slightly inclined so as to keep the material at the corner of the slide. Or we may sublime from a watch glass upon an object slide, as shown in Fig. 125.

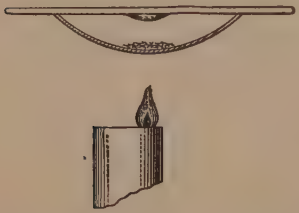

FIG. 125. Watch-glass Method of Sublimation or Distillation.

It sometimes happens that a more crystalline and characteristic sublimation film is to be obtained when the receiving slide is slightly warm, in which event the water is omitted, or, if this is not sufficient, a little cylinder made of carbon, such as is used in arc lamps, is warmed over a burner and placed upon the slide. Such pieces of carbon remain warm for some time and will be found to give excellent results.

Since the temperature and vapor concentration vary over a considerable range, the above method insures that some at least of the sublimates will exhibit satisfactory crystals. If fractional sublimation is to be employed for the separation of two volatile substances, it must be remembered that overheating will raise the temperature of the mixture above the subliming points of both constituents, and they will volatilize together. Very careful heating is necessary in order that the more volatile material may first be driven off, with a minimum of sublimation of the other ingredient. After this is practically complete, the temperature may be gradually raised and the less volatile material sublimed in the later fractions.

With the beginner it is always best to obtain each fractional sublimate upon a separate slide, carefully laying them down film side up in the order in which they have been obtained. Otherwise the films first formed are apt to be driven off by the increasing heat required to vaporize the last portions or will be rubbed off by the fingers or by contact with the support. When a series of sublimation films are obtained upon a single slide the films should succeed each other in such a manner as to bring the first ones farther from the source of heat as each film in turn is formed.

When dealing with sublimations taking place only at temperatures so high that ordinary glass will soften, quartz or pyrex glass slides, nickel or platinum foil, or small nickel or platinum spatulas may be employed. The method of procedure will in any event be similar to that above described, intimate contact between substance and support being first accomplished when possible by moistening with water and careful drying.

Very volatile substances (such as naphthalene), which sublime appreciably at room temperatures, must be examined immediately after condensation in order that their crystals may be observed before they begin to round off and disappear.

Certain substances condense as droplets of supercooled liquid and crystallize slowly or only after stirring or seeding. The possible decomposition of materials, when sublimation is attempted, must always be borne in mind.

The sublimation temperature of a substance is not a constant, but depends on a number of factors. However, the volatilities of materials differ widely, and usually may be represented by a minimum sublimation range for each substance. The temperatures of sublimation may be determined by means of a hot stage such as that described on page 203, or by the method recommended by A. W. Blyth.[18] A small porcelain crucible is nearly filled with mercury, into which dips the bulb of a thermometer. A thin cover-glass, bearing at its center the material to be tested, moistened and dried as usual, is floated on the surface of the mercury. Upon the cover-glass is placed a low glass cell whose upper and lower rims are accurately ground. A second cover-glass is placed above to receive the film (Fig. 126). A number of clean cover-glasses should be placed near at hand. The crucible is heated

Experiment 16. (a) — Sublime and study the following: phthalic anhydride, benzoic acid, tetrachlorbenzene, napthalene, or quinone.

(b) — Fractionally sublime mixtures of any two of the above substances.

(c) — Sublime: indigo, mercuric iodide, or arsenic trioxide.

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[18] Poisons: *Their Effects and Detection* (London, 1906), p. 259.

over the low flame of a Bunsen burner. As the temperature rises, the covers are changed, by means of a pair of forceps, every five or ten degrees. The cover-glasses are examined under the microscope, and a decision made as to the temperature of sublimation. A second and even a third experiment should always be made. If the material fails to sublime at a temperature below that at which the mercury itself is volatilized, a bath of a suitable low-melting alloy must be used. For accurate measurements it is essential to protect the crucible and cell from the cooling effects of air currents.

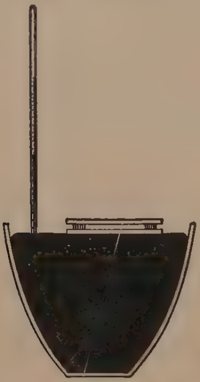
FIG. 126. Micro-Sublimation. Heating by molten metal.

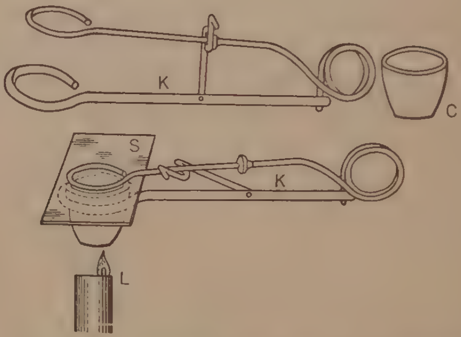

FIG. 127. Apparatus for Micro-Sublimation, Micro-Distillation, and Gas Evolution. K — spring clamp. C — glass crucible. S—object slide. L—micro-burner. (½×).

Subliming upon a glass object slide as shown in Fig. 124 is impracticable when only a minute quantity of the material is available, since the losses through incomplete condensation are considerable. In such an event it is safer to employ the device shown in Fig. 127, primarily intended for distillation but yielding good results with solids as well as with liquids. When, however, only an excessively small amount of material is to be tested, as in toxicological analysis, it is better to drop the substance into a thin-walled glass tube not over 1 mm. in diameter, sealed at one end. Tap the tube gently so as to collect all the material at the sealed end. With a very fine blast lamp flame draw out the open end to a hair-like capillary tube, and, after cooling, gently heat the material in a hot stage of the type shown in Fig. 88, until sublimation takes place. The chief difficulty with the tube method lies in the fact that the poor quality of the glass, the striations, air bubbles, and defects, render the examination of the sublimate complicated and difficult. Laying the tube in a drop of oil or of glycerine at the point where the sublimate appears facilitates the study, by preventing the formation of heavy black contour bands.

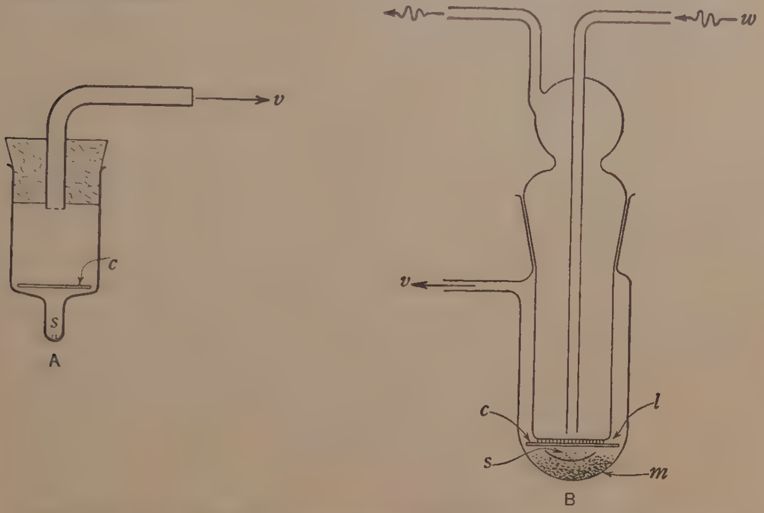

FIG. 128. A — Eder's Apparatus for Sublimation under Reduced Pressure. ($\frac{1}{2}\times$).
B — Werner-Klein Water Cooled Vacuum Sublimation Apparatus. ($\frac{1}{2}\times$).
v to vacuum, *c*, cover-glass on which sublimate condenses, *l* film of liquid, *m* metal filings, *s* substance to be sublimed.

The various types of hot stages described in Chapter VI and the hot plate shown in Fig. 62 are also useful for the control of temperature in sublimation.[19]

Many substances which do not yield sublimates by ordinary methods do so if the surface on which condensation takes place is very close to the material being heated (0.1 to 0.01 mm.), or if the bottom of a vessel containing water is used to condense the vapor.[20] Special types of apparatus have been devised to permit micro-sublimation under reduced pressure, with water-cooling of the surface on which the crystals are deposited.[21] (Fig. 128).

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[19] A sublimation chamber for automatic control of temperature over a period of several hours is described by Ehrismann and Jochimglu: *Biochem. Zeits.* **199**, 272 (1928).

[20] Kempf: *Zeits. anal. Chem.* **62**, 284 (1923).
Kürschner: *Mikrochemie* **3**, 1–20 (1925).

Sublimation temperatures are so dependent upon external conditions that they cannot be observed with the exactness of melting points. Approximate values are frequently of value for purposes of identification, and may be obtained by means of hot stages or the various special types of micro-sublimation apparatus.[22]

Crystallization from Fusion. — Recrystallization from the molten state is an exceedingly useful procedure in the examination of fusible materials, particularly those which are not readily susceptible to other methods of crystallization. Although mainly applicable to the examination of organic compounds, crystallization from fusion is sometimes of value in the study of inorganic substances, such as salts which melt in their water of crystallization. Plant operations involving the solidification of melted material may be advantageously duplicated under the microscope, since the properties of the crystallized product are often closely related to the manner of its formation.[23]

The possible influence of solvents is eliminated by crystallization from fusion, and the purity of the material is often indicated by the sharpness of its melting and freezing. On the other hand, the crystals are usually grown in a thin layer of liquid, and are ultimately in very close contact. Single well-formed crystals are not readily removable for optical tests such as the determination of refractive indices. Fairly close control of temperature is required if the crystal growth is to be slow enough to permit the study of faces and angles or isolated crystals in the melt.

The simplest method of studying crystallization from fusion by means of the microscope is carried out as follows: Place a small quantity of the material in a heap near the corner of a thin slide, and cover it with a cover-glass. The amount used should be such that the melt will spread to fill the enclosed space, with only a small excess at the edge of the cover-glass. Warm gradually by holding the slide several centimeters above the flame of the micro-burner, and gradually increase the temperature by bringing it nearer. Observe the specimen closely, and note any tendency to sublime or decompose. As soon as melting is started, maintain the temperature as nearly constant as possible, to avoid overheating the material, and as soon as fusion is complete withdraw the preparation. Prolonged heating or excessive temperatures are likely to alter the chemical nature of the melt.

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[21] Eder: *Schweiz. Wochschr. Chem. Pharm.* **51**, 228, 241, 253 (1913).
Werner: *Mikrochemie* **1**, 35 (1923).
Klein and Werner: *Zeits. physiol. Chem.* **143**, 141 (1925).
Viehoever: *Jour. Ass. Offic. Agr. Chem.* **6**, 473 (1923).
Hortvet: *idem.* **6**, 481 (1923).

[22] A table of subliming points of a number of substances is given by Mayrhofer: *Mikrochemie der Arzneimittel und Gifte* (Berlin, 1923–28), I Teil, p. 268.

[23] The analogies between microscopic crystallizations from fusion and the casting and structure of alloys are discussed by Chamot and Mason: *Jour. Chem. Education* **5**, 9 (1928). Similar comparisons may be drawn with reference to mineral formation.

The preparation is ordinarily allowed to cool in air, being placed under the microscope in time to observe the progress of crystallization. The faces and angles at the free ends of the growing crystals, the grain boundaries which are the resultant of the growth of adjacent crystals, the formation of air bubbles which are entrained at the grain boundaries as inclusions, the completeness of solidification or the presence of more fusible constituents, and the rupture of crystals by shrinkage as the preparation cools to room temperature, should be carefully studied. Remelting several times is usually essential if all these phenomena are to be observed. Examination between crossed nicols is useful as a means of accentuating the contrast between the crystals and the melt, and recognizing the differences in orientation which correspond to the individual crystal "grains" in the solidified mass. If single isolated crystals occur in the melt, they should be studied with particular care, since they represent the habit of the material better than do the crystals which are restricted by adjacent ones.

If the solidification is too rapid, it may not be easy to follow under the microscope, and the crystal grains produced may be too small to yield interference figures. By cooling very slowly, holding the preparation at some distance above the flame if necessary, and by the avoidance of supercooling, a larger "grain size" may be obtained. If a marked tendency toward supercooling exists, the melt may be "seeded" by the introduction of fresh material or by scratching at the edge of the cover-glass; this should preferably be done at a temperature only slightly below the freezing point. It is often desirable to remelt only a portion of the crystallized layer, by heating at one edge, so as to establish a temperature gradient and to leave some unmelted crystals for seed. The crystallization of substances with low melting points may be accelerated by cooling the slide upon a cold object such as a block of metal.

If more accurate control of temperatures is desired, and if melting points are to be determined, some form of hot or cold stage should be employed (see pages 200, 206).

Experiment 17. (a) — Crystallize the following substances from fusion: thymol (m.pt. 50°), urea (m.pt. 132°), *o*-nitrophenol (m.pt. 45°), or sulphonal (m.pt. 127°), studying as directed above and varying the rate of freezing.

(b) — Fuse some thymol, and color the melt with Bismarck Brown dyestuff. Cover with a cover-glass, and allow solidification to take place, and note that the colored impurity is rejected by the growing crystals, and tends to concentrate in the melt in front of their advancing faces.

Crystallization from the Solid State. — Substances which possess two or more crystalline allotropic modifications exhibit transformations of crystals of one solid phase into those of another, by a process resembling crystallization from the melt. The direct observation of such changes, or of the final structure of the specimen, is readily accomplished by the microscope, and is particularly illuminating in the study of thermal equilibria in one-component systems. The recognition of allotropy is important as a means of characterizing substances, and in explaining and avoiding discrepancies in the determination of the various physical properties of different modifications.

The growth of crystals from a solid substance of different crystalline character is usually manifest under the microscope by the progressive development of a different structure in the specimen. The actual transformation is revealed by a difference in refractive index at the boundary between the two phases, by a change in the double refraction, orientation and other properties, and in many cases by the faces and angles of the growing crystals. Since the original material is solid, with fissures, inclusions, and other discontinuities, no transfer of matter can take place, and these features remain in the secondary crystals, which often exhibit a pseudomorphic appearance, and a smaller "grain size" than the primary crystals.

In the observation of crystallization from the solid state, the material is fused and crystallized as directed above; its cooling is followed under the microscope with ordinary light and between crossed nicols, and also by means of the naked eye.

Ordinarily, the transformations of the different modifications take place more or less spontaneously and require little encouragement unless the cooling has been very severe, when suspended transformation may occur on account of the slowness with which the atoms rearrange themselves into a new space lattice. Seeding or scratching the edge of the preparation will often initiate the growth of the stable phase. Cautious warming often aids in accelerating the change, provided the temperature of reversal is not reached. In general, the transformations exhibited by a substance occur in a sequence, the least stable phase being formed first, and followed by successive transitions, each to a more stable modification.[24]

Two important and common types of transformations between solids are known, enantiotropy and monotropy.[25]

Enantiotropic transformations occur on cooling the substance, and are reversed on heating it. A definite equilibrium temperature exists between the two modifications, and may be determined under the microscope very exactly by noting the temperature at which they can coexist, by means of a hot stage.[26] Any one of the allotropic forms may be a stable phase, depending on the temperature.

If a temperature gradient is established in the preparation, by localized heating, the different phases may exist in different parts of the crystalline layer, and by heating or cooling may be made to advance or recede in accordance with the temperature at different portions of the slide.

Experiment 18. — Study the transformations of one of the following substances, noting the reversal on heating: carbon tetrabromide, ethylamines hydrochloride, potassium nitrate, silver nitrate, mercuric iodide, sulphur; thallous nitrate (3 allotropic modifications); ammonium nitrate (4 allotropic modifications above room temperature).

Monotropic transformations occur only in case an unstable solid phase has separated from the melt, as is likely to be the case if supercooling takes place. This phase is not stable at any temperature below the melting point (at ordinary pressure), and when scratched or seeded tends always to revert to a stable modification, the transformation being completely irreversible. No equilibrium is possible between the two phases, and no transition point can be determined. The unstable phase can be formed only from the melt, or from a less stable phase if several monotropic forms exist.[27]

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[24] In accordance with the law of successive reactions.
Ostwald: *Principles of Inorganic Chemistry* (4th Ed.), p. 235.

[25] Findlay: *The Phase Rule* (London, 1927), p. 35.
Lehmann: *Molekularphysik* (1888–89), Bd. I, pp. 119–219.

[26] The transformations of ammonium nitrate have been studied in this manner by Bowen: *Jour. Phys. Chem.* **30**, 721 (1926).

Monotropic substances may occur in unstable form on ordinary crystallization from the melt, but in testing for the existence of this type of allotropy it is best to supercool strongly and to avoid any possibility of seeding with the stable modification. If the crystals obtained transform on scratching or seeding, and on being heated this transformation is not reversed, monotropy exists. If localized heating is employed, so that a temperature gradient is established in the preparation, the stable phase will always grow at the expense of the metastable one, and the latter will melt at a lower temperature. This may be demonstrated by touching the cover-glass with a hot wire, so as to fuse a small portion of the specimen. Suspended transformations are not uncommon in monotropic systems, and gentle warming will accelerate changes which might otherwise be unobservable.

Experiment 19. — Crystallize the metastable allotropic modification of one of the following and study its transformation, noting the irreversible character of the change: mononitronaphthalene, cinnamic acid, hydroquinone, resorcin, α-monochloracetic acid (3 allotropic modifications; the first transformation occurs spontaneously or on scratching; the second, on seeding with the stable phase).

Allotropy may also be evident when crystallization occurs from solution or from the vapor state. In general, the less stable phase tends to separate first, and later transforms to the modification which is stable under the existing conditions. In the presence of a solvent this transformation is often so rapid as to be overlooked; it tends to be delayed if the crystals are not in contact with each other and if they are continually growing.

Experiment 20. (a) — Recrystallize potassium nitrate rapidly from hot solution and examine immediately for rhombohedral crystals of the metastable form. Observe the rapid transformation of these to the rhombic variety.

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[27] The monotropic transformations of α-monochloracetic acid have been studied microscopically by Mier and Isaac: *Phil. Trans. Roy. Soc.* **209**, 337 (1909). Menthol has been similarly investigated by Wright: *Jour. Amer. Chem. Soc.* **39**, 1515 (1917).

(b) — Recrystallize sulphur from aniline by cooling, and observe the transformation of the monoclinic crystals which first separate, to the stable orthorhombic modification.

(c) — Sublime mercuric iodide, and note the yellow crystals which condense. Examine them at intervals, and observe their transformation to the stable red variety.

Recrystallization in solids may also occur as a result of "annealing," without any phase transformation. This is of particular importance in controlling the mechanical properties of cold-worked metals and alloys.

Experiment 21. — Recrystallize *p*-dichlorobenzene from fusion, and follow its allotropic changes. When the stable modification has been obtained, deform it strongly by pressing on the cover-glass with a blunt instrument. Examine between crossed nicols and note the "mechanical twins" and "slip lines" which are developed. Then hold the preparation at a temperature just below its melting point for several minutes, and observe the new growth of fine grained crystals from the deformed areas.

Crystallization in Binary and Ternary Systems. — The physical chemistry of two- and three-component systems must be taken into account in many crystallization processes, if the nature of the phenomena exhibited is to be recognized. The microscope affords a simple and direct means of observing the behavior of such systems, and serves as an invaluable check upon other methods of investigation. The less complicated phenomena may be identified readily, and considerable qualitative information may be gained without the necessity of preparing an "equilibrium diagram." As a preliminary to more exact investigations, microscopic examination frequently effects a great saving of time, in indicating the method of attacking the quantitative study of the system.

Hydrates of different composition may be differentiated from each other and from the anhydrous substance by study of their crystalline form and optical properties. Ordinarily they appear as distinct and well defined phases in the system, and their mutual transformations may be followed microscopically. By controlling temperature conditions the stability of the different forms may be investigated.

Experiment 22. (a) — Recrystallize the following salts, evaporate to dryness, warm, and follow their efflorescence: sodium sulphate, cupric sulphate, or strontium chloride.

(b) — Add an excess of sodium sulphite heptahydrate to a drop of water, and warm quickly. Examine before solution or evaporation is appreciable and note the dehydration under water, to the anhydrous salt.

(c) — Add an excess of anhydrous sodium carbonate to a drop of water, and observe the formation of the decahydrate as the crystals dissolve.

(d) — Prepare a dilute suspension of plaster of paris in water, and cover with a cover-glass. Allow to stand, and examine at intervals for crystals of calcium sulphate dihydrate (gypsum).

Double salts crystallize differently from either of the single salts which yield ions in common with them. The possibility of double salt formation may be tested by attempting to crystallize solutions containing mixtures of both compounds, and noting whether a third phase appears at any composition. If a double salt can exist in equilibrium with a saturated solution of its constituents in nearly the same molecular ratio as its own, it will recrystallize just as does a single salt. If, however, equilibrium with the solution is only possible when an excess of one of its constituents is present, the other constituent will crystallize before the double salt appears. As a result, the composition of the solution will vary until it contains the necessary excess of one constituent, when the double salt will begin to crystallize. Two entirely different crystalline phases may thus separate when a double salt is dissolved in water and recrystallized. In some cases merely placing a crystal of the double salt in water will cause a separation of one constituent, before solution is complete.

Experiment 23. **(a)** — Recrystallize $2KCl \cdot CuCl_2 \cdot 2H_2O$ from water, avoiding overheating. Compare the crystals obtained with those of potassium chloride and cupric chloride.

(b) — Add an excess of $KCl \cdot MgCl_2 \cdot 6H_2O$ (Carnallite) to water, and observe the separation of crystals of potassium chloride. Evaporate the solution, and note that the double salt (anisotropic) appears just before dryness is reached. Repeat the crystallization, having a large excess of magnesium chloride present to insure that the double salt will be the first phase to crystallize from the solution.

(c) — Precipitate lead iodide, by adding a crystal of potassium iodide to a drop of a fairly concentrated solution of lead nitrate. Note that the precipitate is soluble in excess of potassium iodide solution, and that on further evaporation prismatic crystals of the double salt $KI \cdot PbI_2 \cdot 2H_2O$ separate.

Solid solutions or " isomorphous mixtures " crystallize from solution as discussed on page 334, only one type of crystals being obtained through a considerable range of compositions.

Mixtures of salts may recrystallize to yield both constituents. There may be a mutual effect upon the habit of the crystals obtained, without chemical interaction. However, in many cases an ionic interchange may occur and other possible phases may separate, depending on the relative concentrations in the solution. Such systems are generally difficult to interpret by microscopic examination, although the crystals actually separating can be identified.

Binary systems crystallizing from fusion may exhibit a number of different phenomena, and are particularly instructive because both temperature and composition gradients may be established. By melting the two constituents beneath opposite sides of the cover-glass, heating so as to fuse them simultaneously if possible and to produce a zone of mixing, the entire range of compositions may be represented in the space of a few millimeters. The properties of this system may then be studied, and the thermal behavior of various mixtures compared. If desired, separate preparations of definitely known compositions may also be examined.

Solid solutions show no discontinuity in crystal growth at the zone of mixing. Crystals of either constituent may extend across the preparation, growing by material acquired from any portion of the melt. Only one phase is present, though this may vary in composition in different parts.

Experiment 24. (a) — Crystallize nickel nitrate hexahydrate and cobalt nitrate hexahydrate separately, by fusion. Then place a fragment of each about 1 cm. apart on a slide and cover with a cover-glass. Warm carefully, so that the salts fuse and the drops of the two melts just run together. Follow the freezing under the microscope.

(b) — Study similarly the systems: naphthalene — β-napthol; *p*-dichlorbenzene — *p*-dibrombenzene.

Eutectics are formed as heterogeneous mixtures of two crystalline constituents, when substances immiscible in the solid state separate from a binary melt. The composition of the eutectic mixture is definite, and it melts at a definite temperature, lower than that of either phase.[28]

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[28] The true nature of eutectics as *mixtures of two phases* was not realized, and they were thought to be compounds on account of their definite compositions and melting points, until microscopical examination showed the presence of recognizable crystals of both constituents in them. This was first established in the case of the system *potassium permanganate* — *water*, the "cryohydrate" of which was studied by Ponsot: *Bull. soc. chim. Paris* (3), **13**, 312–16 (1895).

The formation of a eutectic between two substances may be recognized by means of a diffusion preparation. The pure components are melted under opposite edges of the cover-glass, and a zone of mixing is formed. On cooling, the constituent of higher melting point begins to crystallize first, and grows toward the portion of the melt richer in the other component, as the temperature is lowered. When the freezing point of the second constituent is reached, it commences to crystallize, growing toward the zone of mixing. These two phases continue to develop, as cooling continues, and are finally separated by a region of the melt where the eutectic composition exists. When this freezes as a fine grained mass the crystals of one or both constituents may frequently be recognized. On reheating, the eutectic melts first, the above changes taking place in reverse order.

Experiment 25. (a) — Study separately the crystallizations of naphthalene (m. pt. 80°) and phthalic anhydride (m. pt. 131°) from fusion. Then prepare a slide so as to present the entire range of compositions, and study the behavior of the system on cooling. Note particularly the formation and appearance of the eutectic (m. pt. 65° C; 29 per cent phthalic anhydride, 71 per cent naphthalene). Remelt the preparation gradually and allow it to solidify again.

(b) — Observe similarly the formation of eutectics in the following systems: naphthalene — sulphonal; naphthalene — *o*-nitrophenol; sulphonal — *o*-nitrophenol; naphthalene — quinone; naphthalene — thymol; quinone — *p*-dichlorbenzene.

Ternary eutectics may be observed microscopically, by melting the three components under different portions of the cover-glass, so that three zones of binary mixing are formed, with a region of ternary mixing in the center. On solidification the pure components separate successively, then the three binary eutectics, and finally the ternary eutectic.

Experiment 26. — Prepare a slide of the following substances, as indicated above: naphthalene, sulphonal, *o*-nitrophenol. Follow the solidification under the microscope, noting the various phases and eutectics which separate. (The ternary eutectic may remain molten at room temperature.)

Binary compounds formed by the fusion of two substances, exhibit characteristic behavior under the microscope. In general, if a diffusion preparation is studied, the compound will be found to have a fairly high melting point, and the two binary eutectics between it and the pure components of the system may be readily recognized.

Experiment 27. — Study the following binary systems, noting the presence of a compound having a higher melting point than the eutectics on either side of it: *p*-toluidine — salicylic acid; *p*-toluidine — benzoic acid.

Identifications and testing for purity are possible on the basis of the above phenomena.[29] If an unknown substance is thought to be identical with a known material, the two may be mixed, and any change in melting point or the presence of two phases on solidification will indicate their disparity. On the other hand, if the " binary " system behaves exactly the same as either pure constituent, the unknown and the standard are probably the same. Comparison of the two materials in binary systems with a third substance will help to confirm or disprove their likeness.

[29] Lehmann: *Die Kristallanalyse* (Leipzig, 1891), p. 9.
Emich: *Berichte* **43**, 19 (1910).

CHAPTER XI

DETERMINATION OF REFRACTIVE INDICES OF LIQUIDS AND SOLIDS

Refractive index is perhaps the most important physical property utilized in microscopic work. It governs the visibility of all colorless and transparent objects (page 75) and is one of the chief considerations in the choice of a mounting medium (page 167). Furthermore, it is one of the few numerical constants which can be determined by the microscope with ease and accuracy, and it constitutes an exceedingly useful criterion in the classification and identification of solids and liquids.

The index of refraction is a constant for any given substance of definite composition, and it is fully as useful as a characteristic in chemical work as melting or boiling points. Its determination often affords ready means of recognition or differentiation of material, and in many instances it is the only simple means at command for identification. Substances may often be tested *in situ*, without separation from extraneous materials. A minute amount of either liquid or solid sample is sufficient, and it may be recovered if necessary.

The microscope is the most convenient instrument for the determination of refractive indices of solids, within the limits of its accuracy. No preparation of the specimen is required, other than recrystallization if possible. Small particles are used, and highly colored materials are therefore usually transparent enough to be studied. Refractive index may be the only optical constant determinable on very minute grains, and is therefore particularly important in the examination of finely powdered samples, such as paint pigments. Separation from foreign matter is frequently unnecessary; tests may be applied to single grains in aggregates, or inclusions in crystals, glass, or plastics. Tiny spheres of oil, water, or air may be differentiated in biological preparations. The selection of mounting media for optimum refraction images is facilitated by determinations of the refractive index of the object to be studied; this is particularly useful in examinations of animal or plant tissues, natural or artificial textile fibers, pigments and fillers, etc. Particles of gums, resins, plastics and other noncrystalline material may be tested, and such information is frequently of value in predicting the opacity of pigments in them. The transparency of certain fillers in rubber or paints depends upon their relative refractive indices, and the light-scattering properties of pigments are also governed by this factor.

The importance of refractive index as a constant in the identification of crystalline substances has already been emphasized; the various tables of properties listed on page 325 are arranged on this basis.[1] In most chemical problems in which the possibilities are limited, one or two determinations of refractive index may serve to differentiate the materials in question (page 326). Ordinarily the observations need not be exceedingly accurate, since other optical properties and microchemical reactions may be used in conjunction with them. In practically all of the applications of optical investigations which are given in Chapter IX, page 328, refractive index is one of the chief properties utilized, and it offers one more numerical constant for use of the chemist.

Determinations of refractive indices of liquids are less appropriately carried out by microscopic methods, since in many instances a relatively high degree of accuracy is required to render the results of value. However, the microscope may be used to good advantage, as a substitute for a refractometer, in cases where well defined differences exist between the refractive indices of the substances in question. It is particularly advantageous as a means of determining the refractive indices of minute amounts of material, or of highly volatile or reactive substances. Liquids above the range of the ordinary refractometers may be readily tested. Hardened oils or gums may also be examined, and very slow changes in refractive index may be followed.

Each of the different microscopic methods of determining indices of refraction described in the following pages is applicable to a wide variety of materials, both liquid and solid.

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[1] Other tables, dealing primarily with refractive indices, have been prepared by

Schroeder van der Kolk: *Zeits. anal. Chem.* **38**, 615 (1899).
Kley: *idem.* **43**, 160 (1904), (alkaloids).
Bolland: *Monatshefte für Chemie* **29**, 991 (1908); **31**, 387 (1910).
Mayrhofer: *Mikrochemie der Arzneimittel und Gifte* (Berlin, 1928), II Teil, pp. 43–56 (alkaloids and drugs).

In addition to the nature of the specimen, two factors govern refractive index:

The wavelength (color) of the light employed affects the numerical value, which is almost always greater for the shorter wavelengths (violet end of the spectrum). The variation in refractive index with wavelength is spoken of as the **dispersion** of the substance. It varies over wide limits, but in general is greater for liquids than for solids.[2]

The temperature also affects the refractive index, particularly in the case of liquids. In general, the index of refraction is lowered by raising the temperature, the amount differing with the substance in question.[3]

IMMERSION METHODS

All transparent or translucent objects, when immersed in liquids, yield images in the microscope which are bounded by dark shadow outlines or color halos (page 76). The width or thickness of these dark or colored boundaries depends upon the magnitude of the difference between the refractive indices of the two phases, upon the dispersive power of each, and upon the hue and direction of the illumination. The shaded outlines appear, whether the external or the internal phase is of higher refractive index, and tend to vanish as the refractive indices approach equality (Fig. 154). If the object and the surrounding liquid have exactly the same refractive index, and the same color, no line of demarcation will be observable, and the object will be invisible. Complete disappearance is not usually possible in practice, since the dispersions of the two phases are rarely identical and objects which are perfectly homogeneous are uncommon. Crystals or other solids usually contain fissures or inclusions which render them visible even when their outline is not discernible.

The immersion methods of refractive index determination are based upon the above phenomena.[4] Given a series of liquids of known refractive indices, the object, or different portions of it, may be immersed in these successively, until one is found in which the shadow boundaries are at a minimum. In like manner, a series of solids of known refractive indices may be used to determine the refractive index of a liquid.[5] Immiscible liquids may be tested similarly.[6] Even by comparison with a single liquid, a rough estimate may be made as to whether the difference in refractive indices is large or small. Crystals almost always have a higher refractive index than that of the mother liquor from which they separate ($n = 1.33 - 1.4+$); so with a little practice the estimation may be expressed numerically to the first decimal place. If materials are continually being examined in the same immersion liquid (as, for instance, minerals in Canada balsam, $n = 1.53$) more accurate estimates are possible.

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[2] The refractive index of solids increases about 0.001 for every 10–20 mμ decrease in wavelength; liquids exhibit about twice as much change.

[3] A table showing changes of refractive index with temperature for a number of liquids is given by Larsen: Microscopic Determination of Non-Opaque Minerals, *U. S. Geol. Survey. Bull.* **679**, p. 15. In general the decrease for 1° C. elevation is about 0.0004.

As an aid to intelligent trial of the series of standards, some means of ascertaining which phase has the higher refractive index is necessary, for the intensity of shading reveals the amount but not the direction of whatever difference exists. Several methods are available for this purpose; they will be better understood if the accompanying simple experiments are performed as a preliminary to them.[7]

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[4] The discovery of the immersion methods by Maschke and later by Schroeder van der Kolk, and various manipulative procedures, are fully discussed by Johannsen:
Manual of Petrographic Methods (1918), Chapter XIV. See also
Wright: *Methods of Petrographic Microscopic Research*, Carnegie Institution Publication **158** (1911), Chapter II.
Nuthoc: *Amer. Mineral.* **2**, 4 (1917).
Spangenberg: *Centralbl. für Min.* **1920**, 352, 406; *Fortschr. Min. Krist. Petrog.* **7**, 3 (1922).

[5] A table of solid standards is given on page 387. A series of glasses, of known refractive indices, is used by de Souza-Brandão: *Centralbl. für Mineralogie* **1904**, 14; these may be purchased from R. Fuess, Berlin-Steglitz.

[6] The "*Schlieren*" method, as described by Emich: *Monatshefte* **50**, 269 (1928), may be employed for miscible liquids also. A drop of the material to be tested is expelled from a micro-pipette into a liquid of known refractive index, observation being made by highly oblique transmitted light. From the character of the shading and striae at the boundary between the two liquids, the sign of the difference in refractive indices may be ascertained, and, by the use of a series of standard liquids, determinations of considerable accuracy are possible.

[7] Gage: *The Microscope* (1925), p. 112.

The material to be studied should always be mounted beneath a cover-glass, in order to insure complete immersion and to retard the evaporation of liquid mounting media. The preparation should be screened against light from above the stage, in order to eliminate confusing reflections.

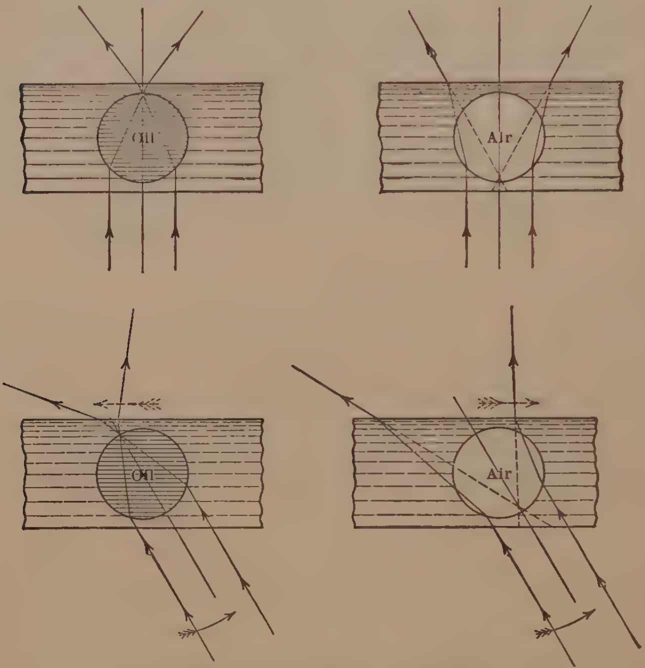

FIG. 129. Oil Globule and Air Bubble, Illuminated with Axial and with Oblique Transmitted Light (Gage).

Focusing upward with axial illumination causes an increase of brightness in the interior of the particle being tested, if it is of higher refractive index than the surrounding medium. The reverse effect is obtained if the particle is immersed in a less refractive medium. This is due to the converging or diverging action on the incident light, as shown in Fig. 129.

Experiment 1. (a) — Place a small drop of mucilage ($n = 1.4 -$) or other viscous liquid on an object slide, and stir air ($n = 1.0$) into it by beating until numerous tiny bubbles are formed. Cover with a cover-glass, and examine one of the smaller bubbles, which is approximately spherical in shape, using an 8-mm. objective and axial transmitted light. The image obtained will be a bright disk of light bounded by a dark circle. Vary the focus upward and downward, and note that the bright center of the image contracts and becomes sharpest at a point below the maximum diameter of the sphere.

(b) — Repeat, using a suspension of oil droplets ($n = 1.4 - 1.5$) in water ($n = 1.33$). Note that the shaded boundary is less bold, because the difference in refractive indices is less than in (a). Vary the focus, and observe that the bright central portion of the image is sharpest at a point above the level of the droplet.

Becke Test. — If the object is bounded by nearly vertical planes, a thin band of light is visible outlining it, when a narrow axial illuminating cone and an objective of low aperture are employed. This phenomenon is called the **Becke line.** The narrow bright halo moves toward the medium of *higher* refractive index, if the focus is *raised*, and toward the medium of lower refractive index if the focus is *lowered*[8] (Fig. 131).

Experiment 2. — Recrystallize sodium chloride ($n = 1.544$), evaporate to dryness, and mount a few crystals in nitrobenzene ($n = 1.55$) or Canada balsam ($n = 1.540-$). Examine with strictly axial illumination, having the condenser lowered and the diaphragm closed, or using the plane mirror alone. Focus up and down slightly, and note the movement of the bright Becke line.

The Becke line test is excellent for tiny grains of material, and for objects bounded by nearly vertical surfaces. It is not always reliable if the object is outlined by inclined or curved surfaces.

Oblique illumination also serves as a test of relative refractive index. If the object is illuminated by unilateral oblique transmitted light[9] it will be unsymmetrically shaded, the behavior being opposite depending on which phase is more highly refractive (Fig. 132). If a particle is surrounded by a medium of lower refractive index, it will appear shaded on the side opposite that from which the oblique light comes, whereas if the surrounding medium is of higher refractive index the reverse will be true.[10] This is due to the converging or diverging effect upon the incident light. The particle may be considered to act as a rough lens, the focus of which will be either above or below its general level. If the illumination is rendered oblique, the bright center of the image will move as indicated in Fig. 129 and in the microscope this motion will appear reversed. One side of the particle will tend to transmit light in such directions that it can enter the objective, and this side will appear bright. The directions of the light rays are represented, greatly exaggerated, in Fig. 130. The crystal *H* has a higher refractive index than the surrounding medium *F* and renders the light convergent so that its left side appears bright and the right dark. In the case of the crystal *L*, the divergence of the light rays is such that its right side appears bright and the left dark.

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[8] Johannsen: *op. cit.*, p. 271.
Wright: *op. cit.*, p. 95.

[9] The various methods of obtaining oblique illumination are discussed on pages 82, 85.

[10] Johannsen: *op. cit.*, p. 256.
Wright: *op. cit.*, p. 92.

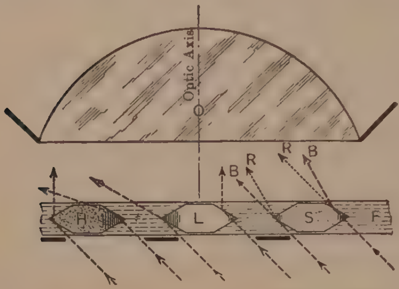

Fig. 130. Crystals with Oblique Illumination, in the Immersion Method of Testing for Refractive Index.

If oblique light is obtained by swinging the mirror, the bright center will appear to move in the same direction, if the particle is of higher refractive index than the surrounding medium.

If a condenser is focused on the preparation, and one side of its aperture screened, that side of the field will be darkened.[11] A particle examined under these conditions will appear shaded on the *same* side as the shadow in the field, if it is of *higher* refractive index than the surrounding medium. If the condenser is lowered, so as to focus much below the level of the object, the above effect is reversed and is less pronounced.

The tests with oblique illumination are much better defined if an objective of low aperture is used, since the more inclined rays from the object are sharply excluded, even when convergence or divergence is slight. The objective with iris diaphragm, described on page 16, is excellent for use in the study of small particles.[12] In any case, the phenomena are best observed by

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[11] For this reason the procedure is sometimes called the "half-shadow method."

[12] Wright: *Jour. Washington Acad. Sci.* **4**, 389 (1914) recommends the use of a second screen in the back focal plane of the objective on the side opposite that below the condenser, as a means of increasing the sensitivity of the tests. This is essentially the principle of the "*Schlieren*" method described by Emich: *Monatshefte* **50**, 269 (1928), and Metzner: *Das Mikroskop* (Leipzig, 1928), p. 278.

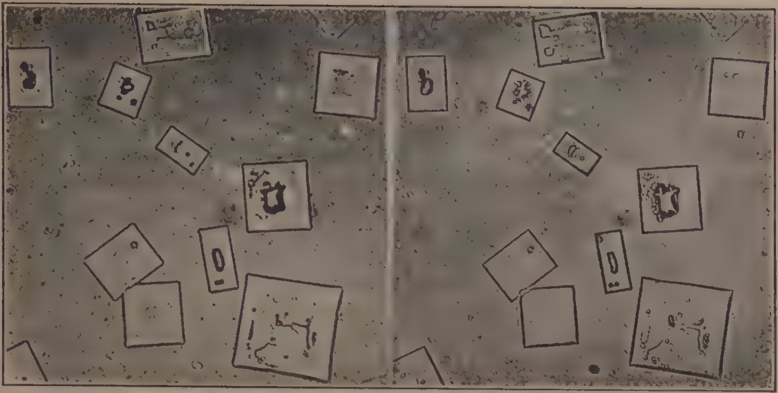

FIG. 131. The Becke Line. Sodium Chloride ($n = 1.544$) in a Liquid of $n = 1.540$.

A — on focusing upward slightly. B — on focusing downward slightly.

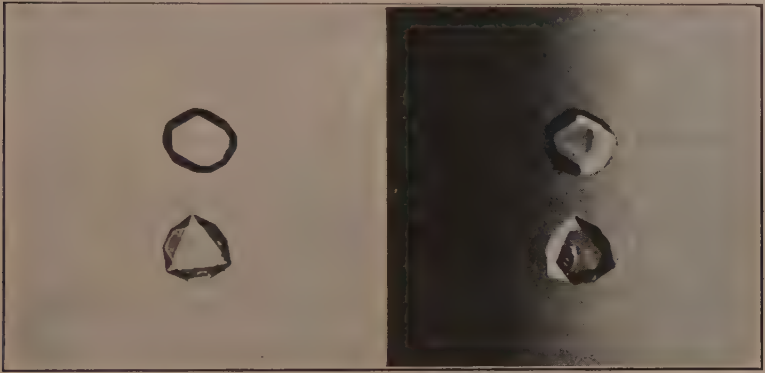

FIG. 132. Axial and Oblique Transmitted Illumination. The upper crystal has a greater refractive index than the mounting medium; the lower crystal, a less refractive index.

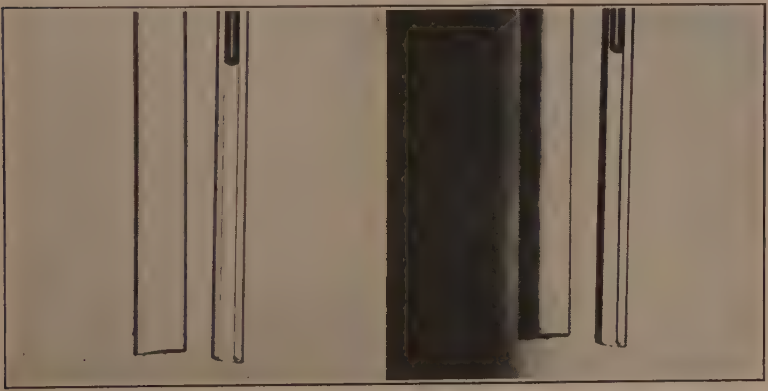

FIG. 133. Axial and Oblique Transmitted Illumination. Glass Rod and Tube in a liquid of lower refractive index which has partially filled the tube.

passing from axial to oblique illumination while watching the changes in the shading of the object.

Experiment 3. (**a**) — Examine the air bubbles and the oil drops of Experiment 1, (*a*) and (*b*), using a 16-mm. or 32-mm. objective. Note the appearance with axial light from the plane mirror, and the direction of the changes in the shading as the mirror is swung to one side.

(**b**) — Study the above preparations with axial or symmetrically by convergent light obtained by means of a condenser focused on or above the preparation. Render the illumination oblique, by shading one side of the fully opened condenser diaphragm with the finger or a piece of paper, and note the shift of the shading with reference to the dark side of the field. Next lower the condenser and observe that the unsymmetrical shading is reversed.

(**c**) — Recrystallize barium nitrate ($n = 1.57$), evaporate to dryness, and mount in liquids of refractive index slightly above and below that of the crystals. Examine with axial and with oblique light, as in (**a**) and (**b**).

(**d**) — Examine a very fine glass rod ($n = 1.5 - 1.6$) dry, and in a liquid having a refractive index near that of the glass, using first axial and then oblique illumination.

(**e**) — Study as in (**d**) a tiny glass capillary, which has been sealed at one end to prevent its becoming filled with liquid. Pay particular attention to the difference between the filled and empty portions of the tube. Note the changes of shading in the walls and in the interior of the tube, and correlate them with the relative refractive indices of each portion.

The tests with oblique illumination are highly sensitive, and are particularly useful in comparisons of the refractive indices of a number of different particles in the same field.

Methods of Applying Immersion Tests. — Solid material should be recrystallized if possible, to guard against its being tested in an effloresced or otherwise altered condition. The last trace of solvent used in the recrystallization should be evaporated, in order to avoid falsifying the tests. In the case of very soluble substances, the bulk of the mother liquor may be drained off by a bit of filter paper as soon as a good crop of crystals is formed, to minimize the incrustation of formless material which obscures the crystals as evaporation proceeds. A sufficient quantity of the sample may be recrystallized at one time to provide for a number of tests, and portions of it may be transferred to several different slides by means of a glass rod or tiny spatula.

Perfect crystals are not necessary, but aggregates and fragments are undesirable. Only particles of recognizable form should be studied, to avoid confusion with dust, lint, or fragments of glass which may be present, and to permit observations of the optical orientation of anisotropic materials.

The particles to be tested are placed on a clean slide and covered with a clean cover-glass. A drop of liquid of known refractive index is introduced at the edge of the cover-glass and allowed to spread beneath it, so as to immerse the particles completely. The liquid should be exposed to the air as little as possible, in order to avoid any change in its refractive index. The preparation should now be examined under the microscope, and well formed crystals selected for study, and placed in the center of the field.

If **isotropic material** is to be tested, axial light from the plane mirror, or the condenser with diaphragm closed, is obtained and the Becke test applied by focusing up and down. Next the tests with oblique illumination are carried out, preferably with an objective of low aperture. The mirror may be swung to one side, or the condenser aperture screened; if the condenser is used, it should be fully raised, so as to avoid the risk of reversed tests. All three sets of observations should be in accord, though the sharpness of the phenomena may differ.

In determining an unknown refractive index it is advisable to start with a liquid of n = *circa* 1.55, since this lies approximately in the middle of the range of possibilities. Not only should the specimen be tested to ascertain whether its refractive index lies above or below this value, but an estimate should be made as to the magnitude of the difference. The next liquid used should be chosen in accordance with this estimated value, and a second test made of the direction and amount of the difference between unknown and standard. Ordinarily less than ten trials are required, and with practice in estimation less than half this number are usually sufficient. Since only a few tiny particles are required for each test, it is best to use fresh material with each liquid. However, it is possible to rinse off the specimen, and use it for a succession of tests.

When the unknown shows minimum visibility in a liquid of known refractive index, a final estimate should be made as to how much its index of refraction is above or below that of the nearest standard. Since most liquids employed have a greater dispersive power than the solids which are tested, at the end-point in the immersion methods the image generally appears surrounded by color halos.[13] The conditions which usually obtain are that when the liquid and the solid have the same refractive index for the middle of the spectrum (yellow, green, for which the eye is most sensitive) the liquid will have the higher refractive index for blue light, and the solid the higher for red light. As a result, the particle will tend to converge red rays and diverge blue, as shown in Fig. 130, *S*. No dark shadow boundary will be sufficiently prominent to be noticeable, but the image will exhibit a bluish fringe on the outside and a reddish one on the inside. With oblique light opposite edges will be tinged with these colors.

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[13] The Christiansen effect, described on page 194.

The exhibition of a marked color fringe at the boundary of the image of the specimen is evidence that the unknown and the standard liquid have the same refractive index, for light of medium wavelength. If more accurate results are required, recourse must be had to monochromatic light.

The accuracy of the Becke test and that of the test with oblique illumination are substantially equal, and the tests are used more or less interchangeably in practice. They serve as valuable checks upon each other, and occasionally the conditions are such that one is better defined and more conclusive. The immersion methods will ordinarily serve to reveal differences of refractive index rather less than ±0.005, depending chiefly upon the dispersive power of the liquid, which should preferably be little more than that of the solid if excessive and confusing color halos are to be avoided. By the use of monochromatic light[14] and controlled temperature[15] an accuracy of ±0.002 or even ±0.001 may be reached.[16] Determinations may be carried out on very minute particles of materials, a few microns in diameter. If smaller grains (such as pigments and fillers), which are barely resolvable, are to be tested, the phenomena may be less easily recognized, but reliance can always be placed on the disappearance of colorless particles if they are surrounded by a medium of their own refractive index.

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[14] Strictly monochromatic light is hardly necessary, and one of the simpler methods given on page 101 will supply a sufficiently narrow spectral region for almost all ordinary determinations. More exact methods for the determination of refractive index and dispersive power have been developed by Winchell and Emmons: *Amer. Mineral.* **11**, 115 (1926); **13**, 504 (1928).

Winchell: *Elements of Optical Mineralogy* (1928) pp. 215–30.

[15] Fluctuations of temperature by radiations from the illuminant are measured by a thermocouple on the object slide, by Ashton and Taylor: *Amer. Mineral.* **13**, 411 (1928). Gaubert: *Bull. soc. française minéral.* **45**, 89 (1923), varies the temperature of the test liquid, to adjust its refractive index to that of the solid. The methods of Winchell and Emmons (*loc. cit.*) are similar in principle.

[16] Further limitations on the accuracy of the methods are discussed by Wright: *op. cit.*, p. 87.

Anisotropic substances are tested by the methods given above, but since they possess more than one index of refraction only approximate results can be obtained with unpolarized light, especially if the double refraction is strong. The refractive index varies with the direction of transmission and of vibration of the light in the specimen, and both these factors should be specified in connection with the determinations; this necessitates the use of the polarizing microscope and permits the observation of two or three constants for the substance, instead of the single refractive index of isotropic materials.

For most " views " of anisotropic substances two refractive indices are exhibited, corresponding to the two component vibrations of light as it passes through the material (page 277). The refractive index is determined for each component separately, in order that the two constants shall be clearly distinguished.

After mounting in a liquid of known refractive index the specimen is first examined between crossed nicol prisms, the directions of vibration of which are known (page 272) and is rotated to a position of "extinction." The vibration directions of the substance are thus aligned with those of the nicols (page 278). One of the nicol prisms is now removed; the other serves to select one of the two component vibrations of light in the anisotropic material. The tests described above are now applied, the position of the substance and the nicol being maintained fixed.

If the microscope is equipped with a condenser above the polarizer, the analyzer may be removed, and either the Becke test or that with oblique illumination may be applied, just as in the case of isotropic materials. If convergent polarized light is not obtainable by means of a condenser, the mirror may be swung slightly sidewise, or one side of the aperture of the polarizer may be screened, to give oblique illumination. Since the illuminating apparatus of polarizing microscopes is sometimes constructed so that anomolous results are obtained, these tests should first be tried on substances of known refractive indices.

Instead of removing the analyzer, the polarizer may be removed, and oblique illumination may be obtained by swinging the mirror, or by means of an ordinary condenser.

The Becke test may be carried out with either of the nicols removed. It is preferable for use with strongly birefringent materials, since it utilizes axial light; highly oblique illumination may give results with such substances corresponding to a slightly different orientation of the specimen.

The relative orientation of the various particles used, with reference to the plane of vibration of the nicol prism, is kept the same for the successive tests with various standard liquids. In this manner the refractive index for one of the two component vibrations is determined.

The above operations and tests are now repeated, this time with the substance oriented 90° from the previous position, so that the refractive index for the other component vibration is observed.

If more than one " view " of the material is observable, the direction of light through it will be different, and other refractive indices may be exhibited by the two components present. Both refractive indices should therefore be determined as described above, for each different " view " of the specimen. Duplications are easily recognized, and by rotating the stage or the nicol 90° two tests may be made for each preparation. Throughout the entire series of observations the orientation of the specimen must be kept clearly in mind, either by means of its external form or by its interference figures. The results are best recorded by means of diagrams (Figs. 120, and 122).

The refractive indices determined for various orientations of an anisotropic specimen do not necessarily correspond directly to ϵ and ω, or α, β and γ (page 299). If the form of the material is definite and consistent enough so that they may be duplicated, such data are highly valuable for purposes of identification, and are often more easily obtainable than the above constants. Whenever possible, however, the refractive indices used by crystallographers should be determined, in order that the published tables of optical properties may be utilized.[17]

This involves studies of the material in orientations which are not necessarily limited to those presented by crystals growing on a microscope slide. To obtain the required diversity of orientations, the particles to be tested should be roughly equidimensional if possible, and should preferably be mounted in viscous test liquids. By moving the cover-glass they may usually be caused to roll about so as to present the different views which exhibit the principal refractive indices of the substance.

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[17] The methods of determining these indices of refraction are discussed in detail by Larsen: Microscopic Determination of the Non-Opaque Minerals, *U. S. Geol. Survey. Bull.* **679** (1921), p. 22.

If possible, the uniaxial or biaxial character of the specimen should be decided from an interference figure or from its geometrical symmetry. Knowledge of the sign of double refraction and of elongation, and of the optic axial angle 2 V are also of value as checks on the quantitative determinations of refractive indices.

The relationships of its vibration directions to its crystallographic axes, as summarized on pages 311–315 will aid greatly in the choice of suitable crystals for testing, and will help to insure that determinations are made on properly oriented material.

If the material is uniaxial, the refractive index ω will be observable whatever its orientation, and may be recognized as such by its occurrence in all determinations of the two refractive indices for different views of the specimen. Only the one index of refraction, ω, is exhibited by isotropic views of a uniaxial substance, in which the light travels along the optic axis (c axis). Of all the other refractive indices determined for different orientations, that most widely different from ω is ϵ. The two principal refractives, ω and ϵ, are directly observable on grains which show maximum double refraction (light traveling perpendicular to the c axis).

If the material is biaxial, the orientation which exhibits maximum double refraction will serve for the determination of γ and α. These refractive indices may also be recognized by the fact that γ is the highest and α the lowest value obtainable from any possible orientations of the substance. Particles which show minimum double refraction, in which light travels along an optic axis, will exhibit the refractive index β. If an interference figure is observable, β may be determined as the refractive index for vibrations perpendicular to the plane of the optic axes.

As a general procedure, the particles may be oriented wholly at random, and the highest and lowest possible refractive indices determined. If either of these appear in all orientations, the material is uniaxial, and this refractive index should be designated ω; the other will then be ϵ. The refractive index for an apparently isotropic particle should be determined; if this is the same as either the maximum or minimum values, it is ω. Otherwise it is β; the highest refractive index is γ, and the lowest is α. If an interference figure can be obtained, the proper designations of the different values may be easily ascertained.

In tables of refractive indices of anisotropic substances, the values ω or β are commonly the basis of arrangement of the substances listed. Since these refractive indices are easier to determine than the others, such a classification may be utilized even if ϵ, or γ and α are not known precisely.

The refractive indices of anisotropic substances should be consistent with their other optical properties, or the accuracy of the various determinations is open to question. For instance, if a material is found to be optically positive from its interference figure, then necessarily $\epsilon > \omega$, or $(\gamma - \beta) > (\beta - \alpha)$ (page 299). If the double refraction is strong, as shown by polarization colors, at least two of the refractive indices must be markedly different from each other (page 281). If $2\ V$ is small, two of the refractive indices must be nearly the same, and distinctly different from the other.[18] The stronger the double refraction, the more accurate must be the orientation in order to obtain accurate values of the refractive indices.

Standard Liquids for the Immersion Methods. — A series of liquids of known refractive indices is essential for the immersion methods. The liquids will also be found useful as temporary mounting media for various materials encountered in microscopic work, where the refractive index of the mountant is of importance in interpretation of appearances and regulation of contrast. Ordinarily the liquids should differ in refractive index by about 0.010, but an interval of 0.005 is preferable for more exact work. Liquids of even closer refractive indices may be useful for precise determinations in a limited range. A series of standards should comprise liquids having refractive indices from 1.360 to 1.780, and extending above this if possible. For studies which involve differentiations between a limited number of substances, a much less extensive series may be adequate.

Since the standard liquids cannot be continually tested, permanence of refractive index is highly important. For very accurate work the value should be redetermined at the time the liquid is used, though this is ordinarily unnecessary with suitable liquids. The liquids should possess no solvent action on the substance to be tested; this may require duplicates which are chemically different, in order to deal with various types of materials. Decomposition or the possibility of chemical interaction with the substances tested is also objectionable. Low volatility, absence of color, and relatively high viscosity are usually desirable. Extreme dispersive power is likely to interfere with the determinations, unless monochromatic light is employed. Mutual miscibility of the different liquids of the series is sometimes convenient, for making up liquids of intermediate refractive indices.

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[18] The computation of $2\ V$ from γ, β, and α is discussed by Larsen: *op. cit.*, p. 10.

In order to make up a series of standard liquids, some mixtures are unavoidable, especially if the interval is 0.005. The volatilities of the ingredients should be approximately equal, or else very slight, in order to minimize the risk of a change in composition by evaporation, either in storage or during the test.[19] Definite chemical compounds or purified oils should be used for as many members of the series as possible. In preparing and standardizing the test liquids, their refractive indices should always be determined by a refractometer, since agreement with published values is only approximate in most cases.[20] They should be checked occasionally, especially in the case of mixtures which are likely to change in composition, but variations of 0.005 in the course of a year are uncommon if the liquids have been properly protected.

Liquid standards for refractive index determinations are best kept in brown glass bottles, of the type shown in Fig. 65. The pipette is a great convenience in handling the liquid, and the ground cap prevents evaporation and accumulation of dust. The bottles may be stored in blocks or boxes with holes bored to receive them, so that a set of standard liquids occupies little space.

The series of liquids given in Table III, page 385, are suitable for standards to be used in the determination of the refractive indices of inorganic substances.[21] Since most of these are solvents for certain classes of organic compounds, various alternative series are useful. If aqueous liquids are desired as standards, solutions of potassium mercuric iodide of various concentrations will cover the range up to 1.73; in glycerine solutions 1.79 may be reached. Potassium iodide or quinoline in glycerine are also useful for testing substances which are soluble in ordinary organic solvents but not in water.[22]

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[19] In making up mixtures from liquids of known refractive indices, the formula $n\ (V_1 + V_2) = n_1\ V_1 + n_2\ V_2$ will aid in estimating the volumes to be employed. The refractive index of the mixture must be measured, however, for ordinarily the computed value is only roughly correct.

[20] The Abbe refractometer is most convenient for measuring refractive indices not exceeding 1.71. An ordinary Pulfrich refractometer, with highly refractive prism, will serve for liquids of $n \leqq 1.88$, but the mixtures used are likely to attack the glass of the prism. The Pulfrich refractometer "with variable refraction angle" (Zeiss) has no upper limit, and is very useful in standardizing highly refractive liquids. The microscopic methods described on pages 367, 380 are useful for media having very high refractive indices, and their accuracy is consistent with the final results obtainable.

[21] Exceptionally complete lists of media for refractive index determinations are given by Johannsen: *op. cit.*, pp. 259–65. See also Larsen: *op. cit.*, pp. 15–20. Media of high refractive index are discussed by Larsen in the above work, and also by Merwin and Larsen: *Amer. Jour. Sci.* (4) **34**, 42 (1912); Merwin: *Jour. Washington Acad. Sci.* **3**, 35 (1913).

METHODS BASED ON DISPLACEMENT OF IMAGE

When an object is viewed through a medium the surface of which is normal to the line of vision, the image observed will appear to lie in a plane above that of the object, the amount of displacement being dependent on the thickness and refractive index of the interposed layer of material. This phenomenon is the basis of the microscopic methods of determination of refractive index which depend on measurement of the displacement of the image.[23]

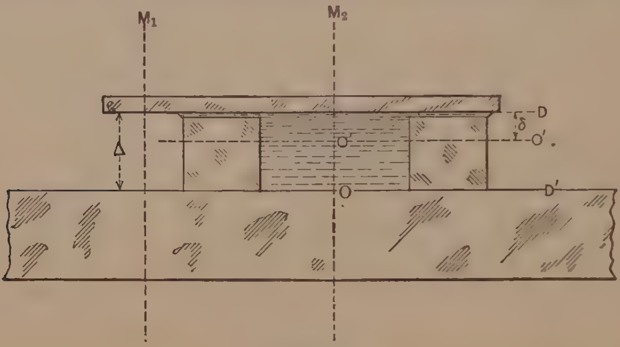

FIG. 134.

The thickness of the layer of material to be tested must be known, or else comparative measurements of displacements must be made with like thicknesses of different media. The former procedure is more suitable for solids, such as chemical crystals or thin sections of minerals. A caliper or dial gage, or the fine adjustment of the microscope, may be used to measure the thickness of the specimen (page 407). If liquids are to be examined, they are usually contained in a cell, the depth of which defines the thickness of the layer.

If a cell of depth DD', Fig. 134, is filled with liquid, the image of the mark O on the upper surface of the slide will be displaced so that refocusing from O to O' is necessary, and the layer of liquid will have an apparent thickness of δ. The refractive index of the liquid is given from the equations

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[22] The refractive indices of a number of media suitable for this purpose are tabulated by Schneider-Zimmermann: *Botanische Mikrotechnik* (Jena, 1922), p. 49. See also Behrens-Küster: *Tabellen zum Gebrauch bei mikroskopischen Arbeiten* (Leipzig, 1908), pp. 48, 50, 52.

[23] This method was devised by the Duc de Chaulnes, and is discussed by Johannsen: *op. cit.*, p. 238, and by Winchell: *Elements of Optical Mineralogy, Part I* (1928), p. 69. See also Addey: *Jour. Queckett Micros. Club* (2) **14,** 279 (1922); Blunck: *Zeits. wiss. Mikros.* **37,** 140 (1920).

$$n = \frac{\text{true thickness, } \Delta}{\text{apparent thickness, } \delta} \tag{1}$$

or

$$n = \frac{\Delta}{\Delta - \text{displacement, } OO'} . \tag{2}$$

The magnitude of the observed displacements, and hence the accuracy of the results, is greater the thicker the layer of material tested. The depth of the cell should approach the working distance of the objective, but should not be so great as to prevent focusing on the bottom of the chamber when empty. An objective of moderately high numerical aperture is necessary for exact focusing. For general work cells designed for use with a 16-mm. objective are most satisfactory; the depth should be about 2 mm., in order that the distances measured through the liquids shall not be greater than the range of the fine adjustment of the microscope. The diameter should be somewhat greater than the depth, to avoid internal reflections from the walls of the chamber. Shallower cells, less than about 1.3 mm. in depth, may be used with an 8-mm. objective.

Cells of the above type may be easily constructed by cementing on an object slide a perforated disk of brass, the surfaces of which are ground true and parallel, so that the top is parallel to the plane of the slide. The cement used must be thoroughly resistant to the various fluids which the cell is to contain, and must not swell or loosen with use. For general work sodium silicate cements are most satisfactory, though not completely proof against aqueous liquids; they should be used sparingly, in order to leave the bottom of the chamber smooth and clean.[24] The cover-glass used should be large enough to extend beyond the edge of the cell.

For filling the cell with various liquids it is best to prepare a number of tiny pipettes by drawing out glass tubing. These may be discarded after use, and will be found particularly convenient for handling volatile or mobile liquids. The chamber should be filled " rounding full " and the cover-glass laid on it and pressed into contact with the upper surface of the cell. Surplus liquid should be avoided if possible, and if any escapes it should be carefully removed with a bit of filter paper, so as to leave the surface of the slide and the projecting cover-glass free from films of appreciable thickness. One or two small bubbles near the edge of the chamber will not interfere with the determination, but larger ones will prevent focusing on the bottom.

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[24] Accurately made cells of welded glass, 0.4, 1.0 and 2.0 mm. deep, may be purchased from Zeiss. They are unnecessarily shallow for use with a 16-mm. objective, but serve very well with higher powers.

In order that the microscope may be readily focused on the bottom and the top of the layer of liquid, the top surface of the object slide and bottom of the chamber may be marked by a scratch or by ink. The under-surface of the cover-glass may be similarly marked, for Method 1. Tiny dust particles which are usually present will also help in focusing sharply on these surfaces.

Instead of marking the level of the bottom of the cell, an image may be projected into this plane by means of the substage condenser (page 43). A scale such as is used for micrometry (page 405) makes a good object for this purpose. The condenser should be used at its full aperture, in order that its depth of focus shall be as small as possible, and the image from it should be carefully adjusted so as to be exactly coincident with the surface of the object slide. The residual color due to the chromatic aberration of the condenser may be utilized to give more accurate settings, by focusing so that the projected image shows a transition tint (say, between red and blue).

Method 1: Cell of Known Thickness. — By making use of equation (1), page 381, the refractive index of a liquid may be determined without the necessity of preliminary preparation of a curve.

Fill the cell, and cover it with a cover-glass, so as to have substantially the conditions shown in Fig. 134. Turn the graduated fine adjustment of the microscope nearly to the lower limit of its range. Place the cell on the stage, and focus on the top surface D' of the slide just outside it, by means of the coarse adjustment, using a scratch, ink mark, or dust particles as a guide. Move the slide so that the projecting cover glass is in the axis of the microscope M_1. Now readjust the focus very carefully by means of the fine adjustment, working through the cover-glass, so that the plane D' is as sharp as possible.

In this and all subsequent settings of the fine adjustment for purposes of measurement, several readings should be taken and the results averaged. For each, the objective should be lowered slightly below the point of focus, and carefully raised until the image is again defined, to avoid error due to lost motion in the fine adjustment (page 407).

If desired, the image of the scale projected by the condenser may now be adjusted so as to be in exact focus in the same plane D'.

Now focus upward by means of the graduated fine adjustment, keeping count of the divisions of the micrometer drum, until the mark on the underside of the cover-glass, in the plane D, is imaged sharply. The final setting should be in an upward direction. Record the scale reading of the micrometer.

The difference between the two readings gives the distance Δ, the true thickness of the layer of liquid.[25]

Restore the fine adjustment to its initial setting, so as to be focused at D' again. Then move the slide so that the axis of the microscope is at M_2. The layer of liquid is thus interposed above the plane D', and the point of focus is thereby lowered. To restore the focus, raise the fine adjustment, meanwhile keeping count of its divisions, until the bottom of the cell at O is sharply imaged. The final setting should be in an upward direction, as before. Read the fine adjustment scale.

The distance traversed, OO', may be subtracted from Δ to give δ, which is the apparent thickness of the liquid, as measured through it. Or, as a further check, the movement upward may be continued to D, the actual distance δ being measured. The refractive index may be calculated by means of the formula $n = \frac{\Delta}{\delta}$.

The above method requires very exact readings, since the values are used directly, without any external check. Unfortunately, most modern fine adjustments are so constructed that the values of the divisions are not absolutely uniform over the entire range of movement, hence measurements of thick layers of liquid are likely to be insufficiently precise. Only under favorable conditions may an accuracy of better than ± 0.01 be expected.

Method 2: Curve Plotted from Measurements of Displacement.[26] — This method depends on the measurement of the amount of displacement of the image by equal thicknesses of liquids of different refractive indices. The results are plotted as a curve, from which the index of refraction of an unknown may be read if its displacement is determined. It is unnecessary to know the depth of the cell, and the fine adjustment need not be perfectly uniform. A cell 3 or 4 mm. deep may be used, with consequent increase in accuracy.

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[25] Measurements of the empty cell by means of a caliper will be slightly less, on account of the absence of the film of liquid beneath the cover-glass.

[26] Suggested to the authors by F. E. Wright, Geophysical Laboratory, Washington, D. C.

Fill the cell and arrange it as directed in Method 1, so as to focus through the projecting cover-glass along the line M_1, on the plane D', Fig. 134. The fine adjustment should be set at the lower limit of its range, so that the same portion of its movement will be utilized for the measurements with each liquid. As directed above, the slack should be taken up, so that no lost motion will occur as the displacement is measured. Record the reading of the fine adjustment micrometer.

Having the objective accurately focused on the plane of the top surface of the slide, move the cell so as to bring M_2 to the axis of the microscope. Measure the displacement through the layer of liquid, by focusing upward, meanwhile keeping count of the divisions of the micrometer. Record the distance, and repeat the entire measurement at least three times, and average the results.

Using the same cell, the same microscope, and the same part of the range of fine adjustment, measure the displacement for at least three liquids having refractive indices which have previously been determined on a refractometer. These known liquids should be chosen so as to give values representing the maximum, minimum, and middle of the range in which future determinations are to be made. For ordinary work water ($n = 1.33$), paraffin oil ($n = 1.47$), and α-monobromnaphthalene ($n = 1.66$) are satisfactory. More liquids may be used, to give a greater number of points on the curve.

Prepare a good-sized graph of displacements against refractive indices. The curve obtained is nearly a straight line, and passes through the point $n = 1.00$, displacement $= 0$.

To determine the refractive index of an unknown, measure the displacement under the conditions employed in preparing the graph, and read its refractive index directly.

The accuracy of Method 2 is greater than that of Method 1, since the use of several sets of readings tends to minimize the error from any one liquid, and serves as a valuable check in the preparation of the curve. Results should be exact to ± 0.005 and with practice even more accurate determinations are possible.

Other methods for microscopic determinations of refractive indices of liquids, besides the immersion methods mentioned on page 367, have been proposed by Wright,[27] who gives an excellent critical discussion of a number of procedures for determining the refractive index of minute amounts of liquid. Most of these require special cells or apparatus which is not easily available, but are worthy of consideration for special investigations.

[27] *Jour. Washington Acad. Sci.* **4,** 269–79 (1914). See also Johannsen: *op. cit.,* p. 265.

Table III

LIQUID STANDARDS FOR REFRACTIVE INDEX DETERMINATIONS BY IMMERSION METHODS

Index of refraction (daylight, 20–22° C.)	Name
1.32	Methyl alcohol
1.333	Water
1.358	Acetone
1.36	Ethyl alcohol
1.38	Ethyl butyrate
1.39	Hexane
1.394	Isobutyl alcohol
1.40	Heptane
1.40	Ethylene glycol monomethyl ether
1.41	Amyl alcohol
1.41	Ethylene glycol monoethyl ether (" Cellosolve")
1.42	Ethylene glycol monobutyl ether
1.44	Chloroform
1.44	Ethylene chloride
1.45	Kerosene
1.46	Carbon tetrachloride
1.47	Cajeput oil
1.47	Glycerine
1.47	Olive oil
1.475	Paraffin oil ("Nujol")
1.475	Turpentine
1.48	Castor oil
1.49	Dibutyl phthalate
1.494	Xylene
1.495	Benzene
1.50	Lubricating oil (for automobiles)
1.515	Anisol
1.515	Cedar wood oil
1.52	Monochlorbenzene
1.53	Clove oil
1.54	Ethylene bromide
1.55	Nitrobenzene
1.56	Tricresyl phosphate
1.56	Monobrombenzene
1.57	*o*-Tuoluidine
1.58	Monobromphenol
1.586	Aniline
1.59	Bromoform
1.60	Cassia oil
1.61	Quinaldin
1.615	Cinnamic aldehyde
1.62	Monoiodobenzene
1.62	Quinoline
1.625	Carbon bisulphide
1.63	α-Monochlornaphthalene
1.66	α-Monobromnaphthalene
1.74	Methylene iodide
1.78	Methylene iodide saturated with sulphur.
1.87	Methylene iodide, S. CHI_3, SnI_4, AsI_3, SbI_3.*
1.64–2.10	Piperine, AsI_3, SbI_3.*
2.0–2.7	Sulphur-Selenium mixtures.†

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* Merwin: *Jour. Wash. Acad. Sci.* **3**, 35 (1913).

Larsen: Microscopic Determination of the Non-Opaque Minerals, *U.S.G.S. Bull.* **679** (1921) p. 15.

† Merwin and Larsen: *Amer. Jour. Sci.* (4) **34**, 42 (1912).

Larsen: *op. cit.*, p. 18.

TABLE IV

LIQUID MIXTURES FOR REFRACTIVE INDEX DETERMINATIONS

Refractive index (daylight, 20–22° C.)	Ethyl alcohol $n = 1.362$	Ethylene glycol monoethyl ether ("Cellosolve") $n = 1.407$	Kerosene $n = 1.443$
1.370	50 cc.	9 cc.	
1.380	50	30	
1.390	35	50	
1.400	10	50	
1.410		50	5 cc.
1.420		50	35
1.430		35	75
1.440		5	75

TABLE V

LIQUID MIXTURES FOR REFRACTIVE INDEX DETERMINATIONS*

Refractive indices	Mixtures
1.450–1.475	Petroleum (kerosene) and turpentine.
1.480–1.535	Turpentine and ethylene bromide or clove oil.
1.540–1.635	Clove oil and α-monobromnaphthalene.
1.640–1.655	α-Monobromnaphthalene and α-monochlornaphthalene.
1.650–1.740	α-Monobromnaphthalene and methylene iodide.
1.740–1.790	Sulphur dissolved in methylene iodide.
1.790–1.960	Methylene iodide, antimony iodide, arsenic sulphide, antimony sulphide, and sulphur.

* Wright: *Methods of Petrographic Microscopic Research*, Carnegie Inst. Pub. **158** (1911) p. 96.

TABLE VI

ISOTROPIC CRYSTALS FOR DETERMINATIONS OF REFRACTIVE INDICES OF LIQUIDS BY IMMERSION METHODS

Refractive index	Name	Formula
1.326	Sodium fluoride	NaF
1.340	Potassium fluosilicate	K_2SiF_6
1.361	Potassium fluoride	KF
1.370	Ammonium fluosilicate	$(NH_4)_2SiF_6$
1.439	Sodium alum	$Na_2SO_4.Al_2(SO_4)_3.24\ H_2O$
1.456	Potassium alum	$K_2SO_4.Al_2(SO_4)_3.24\ H_2O$
1.459	Ammonium alum	$(NH_4)_2SO_4.Al_2(SO_4)_3.24\ H_2O$
1.481	Potassium chromium alum	$K_2SO_4.Cr_2(SO_4)_3.24\ H_2O$
1.488	Ammonium iron alum	$(NH_4)_2SO_4.Fe_2(SO_4)_3.24\ H_2O$
1.490	Potassium chloride	KCl
1.494	Rubidium chloride	$RbCl$
1.504	Sodium uranyl acetate	$NaC_2H_3O_2.UO_2(C_2H_3O_2)_2$
1.515	Sodium chlorate	$NaClO_3$
1.544	Sodium chloride	$NaCl$
1.553	Rubidium bromide	$RbBr$
1.559	Potassium bromide	KBr
1.567	Strontium nitrate	$Sr(NO_3)_2$
1.571	Barium nitrate	$Ba(NO_3)_2$
1.617	Sodium bromate	$NaBrO_3$
1.640	Ammonium chloride	NH_4Cl
1.641	Sodium bromide	$NaBr$
1.645	Cesium chloride	$CsCl$
1.650	Rubidium iodide	RbI
1.657	Potassium chlorostannate	K_2SnCl_6
1.667	Potassium iodide	KI
1.678	Ammonium chlorostannate	$(NH_4)_2SnCl_6$
1.698	Cesium bromide	$CsBr$
1.703	Ammonium iodide	NH_4I
1.755	Arsenic trioxide	As_2O_3
1.774	Sodium iodide	NaI
1.782	Lead nitrate	$Pb(NO_3)_2$
1.787	Cesium iodide	CsI
1.827	Potassium chloroplatinate	K_2PtCl_6
2.06	Silver chloride	$AgCl$
2.25	Silver bromide	$AgBr$

TABLE VII

APPROXIMATE REFRACTIVE INDICES OF MISCELLANEOUS SUBSTANCES

	"ω"	"ε"
" Acetate silk "	1.48	1.475
" Bakelite "	1.58–1.63	
Celluloid	1.53±	
Cellulose fibers (cotton, flax, ramie, etc.)	1.53	1.59
" Collodion silk "	1.52	1.55
Gelatine, dry	1.54	
Horn	1.56±	
Lacquer (" Duco ") dry	1 54	
Linseed oil, dry	1.48–1.50	
Rubber, pale crepe (vulcanized, higher)	1.51+	
Shellac	1.54	
Silk	1.54	1.59
Starch	1.53	
Varnish, dry	1.51–1.54	
" Viscose silk "	1.525	1.55
Wool	1.54+	1.55+

CHAPTER XII

MICROSCOPIC MEASUREMENTS; PARTICLE-SIZE DETERMINATIONS

Measurements of microscopic objects are of widespread usefulness not only for the purpose of determining dimensions but also as an aid to identifications and in the quantitative analysis of mixtures. The size of many natural objects is more or less characteristic, and in the case of textile and paper fibers, starches, micro-organisms, and many other substances, may be very valuable as a means of identifying or of differentiating between materials of similar appearance but different magnitude. The dimensions of artificial materials are frequently of the utmost importance in governing their physical and chemical properties; microscopic measurements are commonly utilized in testing pigments, fillers, abrasives, fibers, ceramic materials and products, protective coatings, metals and alloys, sieves, pulverized ores, and many other kinds of materials where fineness of structure is important.

Units of Microscopic Measurement. — In order to avoid the continual necessity of dealing with decimal fractions, the unit of microscopic linear measure is taken as the micron (abbreviated μ, *mu*), equivalent to 0.001 mm. As a still smaller unit, the millimicron (abbreviated $m\mu$),[1] equivalent to 0.001 μ, may be employed.

Accuracy of Linear Microscopic Measurements. — Apart from mechanical inaccuracy in the apparatus used, there is an inherent limit to the precision with which microscopic dimensions may be measured. The apparent sharpness and fineness of the scale of the micrometer, and of the points on the object between which the measurement is made, are far from mathematically perfect, and the width of the lines which are brought to juxtaposition is very appreciable. The coarseness of the image of the markings on the micrometer scale is governed by their actual fineness, and by the extent to which they are magnified. The outlines and details of the object vary in apparent width, depending on the focus, the illumination, the refractive indices of the preparation, and most of all on the resolving power of the microscope. Only under the best conditions of illumination, with a minimum of diffraction patterns and with delicate, well defined structural details, is it possible to achieve the utmost accuracy of microscopic measurement. The limit is ultimately dependent upon the breadth of the dark diffraction line which outlines microscopic structures, and is approximately equivalent to the resolving power of the optical system.[2] Hence the true position of any boundary which is to be measured is uncertain, to this extent. If an objective of high resolving power is used, the absolute error may be very small; for example, *circa* $\pm$ 0.2 μ for an objective of 1.40 N.A. This may be negligible in measuring the width of a textile fiber which is 25 μ in diameter, but it becomes much more important when pigment particles less than 1 μ in diameter are measured. By averaging repeated measurements the accuracy may be considerably increased,[3] especially if measurements can be taken between centers rather than between edges of the structures.

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[1] Since μ symbolizes a millionth (*cf.* μ gram) the abbreviation $\mu\mu$ for millimicron is ambiguous and misleading; $m\mu$ is used in the International Critical Tables. See Gage: *The Microscope* (1925).

METHODS OF LINEAR MEASUREMENT[4]

All the methods of measuring linear dimensions by means of the microscope depend on the comparison of the image of the object with a scale of known value. In all of them, a microscopic standard of definite dimensions is required for the initial determination. For this purpose some form of **stage micrometer** is used, consisting of an accurately divided scale engraved on a suitable slide of glass or metal.[5]

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[2] Rogers: *Proc. Amer. Micros. Soc.* **1883**, 198.
Nelson: *Jour. Roy. Micros. Soc.* **1903**, 579.
Spitta: *Microscopy* (1920), p. 265.

[3] Ewell: *Jour. Roy. Micros. Soc.* **1910**, 537.
Nelson: *ibid.* **1910**, 696.

[4] Useful discussions of methods and applications of linear measurements are given in the following works:
Gage: *The Microscope* (1925), p. 143.
Metzner: *Das Mikroskop* (Leipzig, 1928), p. 165.
Krause: *Enzyclopädie der mikroskopischen Technik,* II (Berlin, 1926), pp. 1435–42.
Herzog: *Mikroskopische Untersuchung der Seide und Kunstseide* (Berlin, 1924), p. 5.
Johannsen: *Manual of Petrographic Methods* (1918), Chap. XVI.
Lawrie: *Textile Microscopy* (London, 1928), Chap. V.

Method 1: Visual Estimation Based on a Knowledge of the Magnifying Power of the Microscope. — The magnification of the microscope (page 7) is always expressed on a *linear* basis, and is calculated as the ratio of the diameter or length of the image to that of the object. For visual work this image is considered to be 250 mm. from the eye. If the magnification is known, the true size of the object is readily estimated. For instance, if a specimen appears the same size in the microscope as if it were 1 cm. in diameter at a distance of 250 mm., and the magnification is 100×, its true diameter is 0.1 mm.

Measurements based on estimating how large the image appears, assuming it to be 250 mm. from the eye, represent the simplest and least accurate method of micrometry, but are useful as approximations, and serve as a basis for comparison of the sizes of a series of objects all viewed under the same conditions.

If the magnification is not known it may be computed from the magnification numbers or focal lengths of the objective and eyepiece (page 7), or it may be determined more exactly as given below.[6]

Method 2: Measurements Obtained by Means of a Stage Micrometer and a Drawing Camera. — **Drawing cameras (cameras lucidas)** are devices which are attached above the eyepiece of the microscope to permit simultaneous observation of the specimen on the stage and of external objects.

It is very frequently the case that sketches, relative proportions of structural details, or actual measurements of component parts of preparations being studied must be entered into notebooks. Free-hand drawing is tedious and difficult; and, if a sketch to scale is required, as is usually the case, an exceptionally good judgment of proportion is essential. To obviate these difficulties a drawing camera may be employed. Although there are many types of these devices upon the market, the chemist is usually restricted to those forms which permit employing the microscope in a vertical position.

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[5] A critical study of the accuracy of the rulings of stage micrometers has been made by Ewell: *Jour. Roy. Micros. Soc.* **1908.** Stage micrometers may be sent to the Bureau of Standards at Washington, to be tested for precision.

[6] The method of determining the magnification of a projected real image is given on page 406.

The most convenient of these drawing cameras are shown in Figs. 135 and 137. In these types, there is placed above the ocular a cube of glass (Abbe prism) which has been cut diagonally, the surface of one-half being silvered and cemented again in place after a central oval perforation has been made through the silvered surface. This oval aperture allows the image-forming rays of the microscope to reach the eye, while the silvered surface reflects from a mirror the image of the notebook page or drawing paper. Figure 136 shows diagrammatically the path of the light rays, the dotted lines indicating the image-forming rays from the drawing paper *BB* reflected by the mirror *M*, to the reflecting surface *ef* of the Abbe prism *P*, and thence to the eye of the observer. The solid lines indicate the image-forming rays from the preparation upon the stage of the microscope, passing through the aperture in *ef* also reaching the eye. It is obvious that the observer is able to see both the virtual image of the preparation and the drawing paper, and can therefore trace upon the paper with a pencil the outlines and many details of structure of the object.

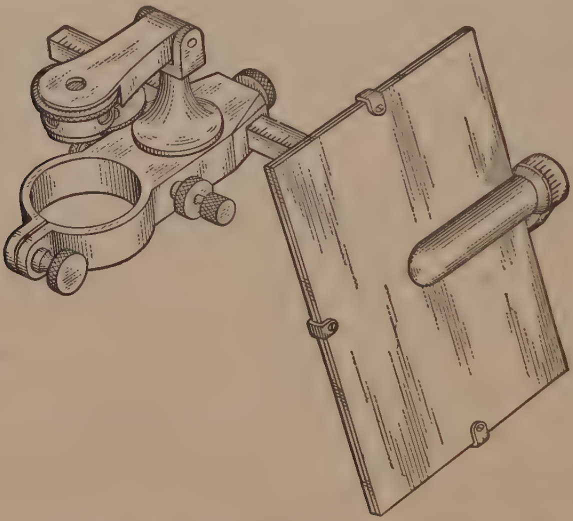

FIG. 135. Large Abbe Drawing Camera. (Spencer Lens Co.)

From an examination of the diagram it will be seen that unless the opening in *ef* is placed at the eyepoint considerable light will be lost and the field will be restricted. Before attaching a drawing camera always first ascertain the position of the eyepoint (see page 30). It not infrequently happens that in designing an ocular, the manufacturer fails to take into account the fact that the investigator may wish to use a drawing camera. The eyepoint may in such cases lie so close to the eye lens or may lie so far above it as to render the employment of an Abbe prism camera impracticable. Because of this great difference in the relative position of the eyepoint in different oculars it is best, in purchasing an Abbe camera, to select one of the type shown in Fig. 135, since in instruments of this sort, the prism mounting is of the smallest dimensions possible and the distance between prism and clamping ring will allow considerable latitude in movements up and down.

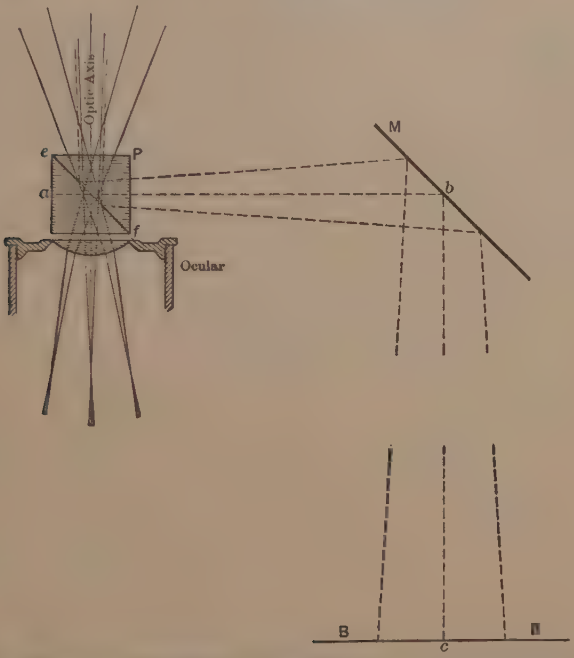

Fig. 136. Diagram of the Path of Light Rays in Abbe Drawing Cameras.

In order to equalize the intensity of the light reaching the eye from preparation and drawing paper, a series of dark glasses of graded transmission are mounted so as to turn into position, by a ring between prism and paper, and a ring between prism and ocular. By properly adjusting the intensity of the illumination of the microscope and then selecting the right glasses in these rings, it is always possible to obtain a clear image of both preparation and drawing pencil.

In order to avoid distortion of the drawing the mirror *M* must be so inclined that the light ray *bc* shall be normal to the paper.

The large cameras of the type just referred to are provided with a graduated extension bar to which the mirror is attached to facilitate adjustments, and the axis upon which the mirror tips is graduated into degrees. When the paper lies horizontally with respect to the optic axis of the microscope, the mirror should be set at 45°, providing that the mirror bar is long enough to prevent interferences due to a reflected image of the stage; if not, then the mirror must be tipped to an angle nearer to the horizontal and the drawing paper inclined until the central rays become normal to it. The amount of inclination of the drawing surface must be twice as many degrees as the mirror is tipped below 45°.

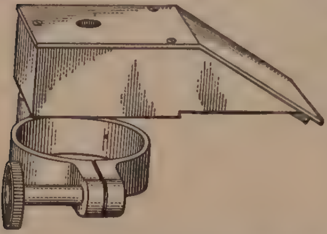

FIG. 137. Small Abbe Drawing Camera. (Bausch & Lomb Optical Co.)

The Leitz Drawing Eyepiece, shown in section in Fig. 138, consists of a negative eyepiece, with lenses so mounted as to permit the insertion of a reflecting prism *P* just above the eye lens and extending to the optic axis of the ocular. Light rays (as indicated by the dotted line) from the drawing paper enter the prism, are twice totally reflected from the inclined surfaces of the prism, and enter the eye together with the image-forming rays of the microscope. The eye therefore perceives the image of the object under the microscope apparently projected upon the drawing paper. Neutral tinted glasses *N* serve to reduce the light intensity from the drawing paper and thus to facilitate following the tracings of the pencil point. The screw *S* serves to clamp the device in place while in use.

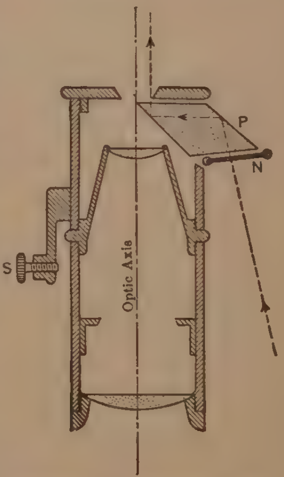

FIG. 138. Drawing Eyepiece. (Leitz.)

Since the prism forms an integral part of the eyepiece, changes in magnification must be made wholly by changing objectives, tube length, or changing the distance from drawing board to prism.

The small drawing cameras shown in Figs. 137 and 138 will give distorted images unless the microscope or the paper is inclined so that the ray from the center of the drawing is normal to its surface. The necessary adjustment must be made by trial.

Cameras lucidas serve not only for drawing but are most useful in micrometry and quantitative analysis,[7] in reading thermometers when melting, boiling or subliming points are determined, or in reading scales of small voltmeters or ammeters when observations are being made, for upon looking into the microscope both the preparation and the scale of the instrument may be seen.

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[7] See Coghill and Bonardi: Approximate Quantitative Microscopy of Pulverized Ores, *U. S. Bureau of Mines Tech. Paper* **211** (1919).

Drawings of complicated preparations are easily made in true proportion, with the aid of the drawing camera. The instrument is adjusted so that the image of the specimen and the notebook page are seen in appropriate size; and the outline of the object is traced. The entire drawing may be executed by this method, but ordinarily only boundaries and the most significant dimensions and structural features are thus recorded. The reflecting prism is then swung aside, and the remaining details are filled in freehand, with better visibility of fine structure than if the images from microscope and notebook were superposed.

Drawings to scale may be prepared according to the above procedure. A stage micrometer is then substituted for the preparation under the microscope, and its divisions are traced on the drawing without altering any of the factors governing magnification. The scale thus drawn indicates directly the various dimensions of the object, and may conveniently be used for **micrometry.** Any detail of the object may be measured at any subsequent time.

If other drawings are to be made to the same scale, the factors governing the magnification should be recorded, and the original conditions duplicated each time. As an alternative procedure, it is possible to make drawings to the same scale by adjusting these different variables until a tracing of the stage micrometer is identical with that prepared previously.

Determination of Magnification by Means of the Drawing Camera. — All that is necessary to determine the magnification of a drawing made to scale by the method outlined above is to measure the actual length of one scale division by means of a ruler, and to compare it with the distance on the scale itself. For instance, if a drawing is made to a scale such that one division of the stage micrometer (0.1 mm.) is represented as 8 mm., the magnification of the drawing is 80×.

The "magnification" of the virtual image is determined similarly, by supporting the drawing paper at such a height as to give a total distance of 250 mm. from the eyepoint to the mirror and thence to the paper, tracing the stage micrometer divisions, and comparing with a ruler. A simpler method is to calculate the magnification on the basis of the actual distance from the eyepoint to the paper. For example, if the magnification of 80× discussed in the preceding paragraphs was obtained at a distance of 400 mm., the magnification at an image distance of 250 mm. will be

$$\frac{250}{400} \times 80, \quad \text{or} \quad 50\times$$

Obviously, by varying the different controlling factors, drawings may be prepared at any desired magnification, and to any scale which may be necessary or convenient.

Method 3: Measurements Obtained by Direct Comparison of Object and Micrometer Scale. — It is rarely possible to observe the object and the scale simultaneously by having them lie in the same plane, though some simple magnifiers are equipped with scales for this purpose. In "micrometer," "traversing," or "reading" microscopes the object or the microscope is moved over the distance to be measured, and the motion is read on a scale; this is, in principle, substantially a direct comparison of dimensions with the micrometer scale.

The construction of typical instruments for this method of micrometry is shown in Figs. 139 and 140. In either one, the microscope is equipped with crosshairs, and the actual movement is measured by means of a graduated micrometer head.[8]

A graduated **mechanical stage**, of the type shown in Fig. 29, is applicable to measurements of objects larger than the field of the microscope. One end of the specimen is placed beneath the crosshairs of the eyepiece, and the scale of the mechanical stage is read accurately by means of its vernier. The specimen is moved so as to bring the other end to the same position, and the scale reading of the mechanical stage is again taken. The difference gives the length of the specimen. For such work it is necessary that the graduations of the mechanical stage be checked directly against an accurately ruled scale, or against a stage micrometer by the converse of the above method.

The diameter of the field of view of the microscope may be determined by a procedure analogous to that given above.

Special mechanical stages, with micrometer heads graduated to measure very small distances of movement, are useful for accurate determinations of dimensions greater than the field of the microscope.[9] If distances are to be measured cumulatively, as in linear analysis (page 445) a special recording micrometer is almost essential. These instruments have a separate micrometer screw and scale for the measurement of each ingredient in the mixture.[10] Of the various models that described by Hunt[11] has the best design and is

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[8] Instruments such as the vertical reading microscope of Leitz operate on this principle, the desired dimension being measured by moving the microscope by means of a graduated upright support, analogous to a cathetometer.

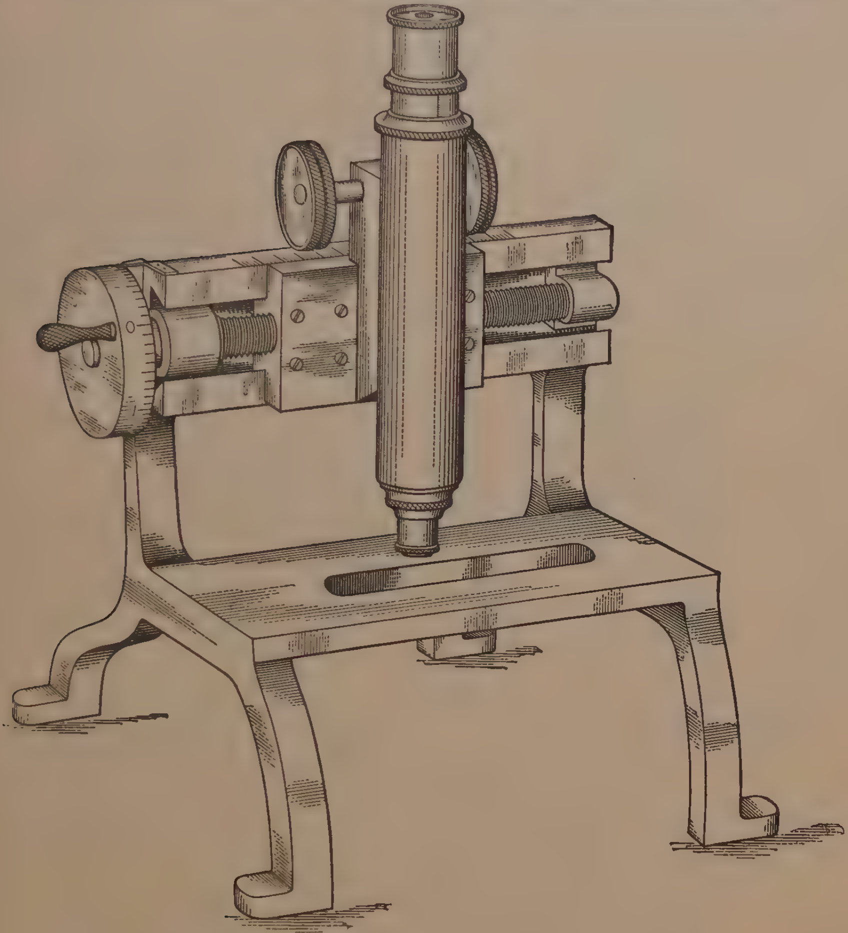

FIG. 139. Micrometer Microscope (R. & J. Beck).

[9] The "stage screw micrometer" of Zeiss permits readings to 2 μ over a range of 10 mm.

[10] Shand: *Jour. Geol.* **24**, 394 (1916).
Wentworth: *idem.* **31**, 228 (1923).

[11] *Amer. Mineralogist* **9**, 190 (1924). Designed by Wentworth and manufactured by Eberbach & Son Co., Ann Arbor, Mich.

most convenient to use. The stage which carries the specimen is attachable to the top of any microscope stage, and its movement is recorded by any one of five coaxial screw heads, each of which is graduated like a micrometer caliper. Linear analyses of mixtures of five components are possible with this apparatus; models with fewer micrometer screws may be used if fewer components are to be measured.

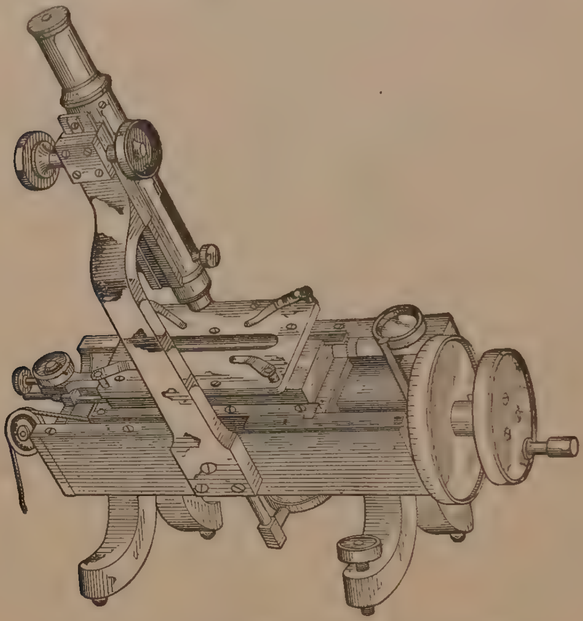

FIG. 140. Micrometer Microscope (Gaertner).

Method 4: Measurements Obtained by Micrometer Eyepieces. — In order to render object and scale simultaneously visible, the scale may be contained in a micrometer eyepiece, at its focal plane. By this arrangement the real image formed by the objective is superposed on the scale, and both are magnified by the eye lens of the eyepiece and seen together in the virtual image.

The number of scale divisions covered by the image gives a value for the image only, but *not* for the object, since the size of the image can be varied without changing the size of the divisions. It is therefore necessary in all cases[12] to ascertain the value of the divisions of the micrometer scale with respect to each objective used, for one or more definite tube lengths.

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[12] Except when employing an eyepiece micrometer which has been ruled by the manufacturer to give a definite value for each division, when used with the particular objective and tube length specified.

Calibration of Micrometer Eyepieces. — Focus the eye lens of the eyepiece so that the graduations of the scale become clear and distinct. Lay the stage micrometer upon the stage and move it until the center of the rulings falls in the optic axis of the microscope; focus carefully and adjust the micrometer until a line becomes coincident with a line of the eyepiece scale. Count the number of divisions of the eyepiece scale included between one or more divisions of the stage micrometer. Divide the value of the stage scale by the number just obtained. The quotient gives the value of one eyepiece scale division.

It is usually the case that conditions obtain giving an appearance as shown in Fig. 141. It is obvious that in such an event it is necessary to estimate the fractional part of a division. Such an estimation or guess introduces a serious error into the method. Moreover, the image of an object to be measured rarely covers exactly a whole number of divisions of the eyepiece micrometer, and one is obliged to make a guess as to what fraction of a part to add. Thus there are two estimates necessary, and any measurements recorded must necessarily be mere approximations. The second of these errors cannot be eliminated in micrometer oculars with fixed scales having rulings of non-variable magnitude, but the determination of the ocular micrometer value may be made more exact by eliminating fractions as shown in Fig. 142. This is accomplished by altering the ratio between the images of the two scales through a change in the position of the draw-tube.

Start with the draw-tube extended about half its total possible movement. Bring the zero point of the eyepiece scale in contact with a ruling on the stage scale; focus sharply. The relations of the images of the two scales will now probably be essentially as indicated in Fig. 141. Note the position of the other end of the eyepiece scale with reference to the nearest ruling of the stage micrometer, and decide whether it will be necessary to enlarge the image or to reduce it, in order to render these rulings coincident. Then make the appropriate change by extending or decreasing the tube length, and note whether it is now possible to have both ends of the eyepiece scale coincide with rulings on the stage micrometer. Repeat the trial until this is the case, or until a *whole number* of divisions of one scale is equivalent to a *whole number* of divisions of the other, as shown in Fig. 142.

It is not essential, and is highly improbable, that the division of the two scales should coincide throughout the length of one of them, but on the basis of the relationship just established it is easy to calculate the value of either in terms of the other.

For convenience in future measurements it is always worth while to obtain such a ratio between the two scales that the calculated value of the eyepiece micrometer divisions will be a simple integral number. This may be accomplished by a few trials at different tube lengths.

With ordinary objectives of comparatively low powers, the use of a tube length slightly different from that for which the lenses are designed affects their corrections so little as to be negligible in micrometry.

In order that the conditions may be duplicated under which the eyepiece micrometer value has been obtained, it is obvious that a record must be made of the draw-tube length employed; the notebook entry will therefore take some such form as this:

> 16-mm. objective, draw-tube 175; 50 divisions eyepiece micrometer = 8 divisions stage micrometer = 0.80 mm.; 1 division eyepiece scale = 0.016 mm. = 16 μ.

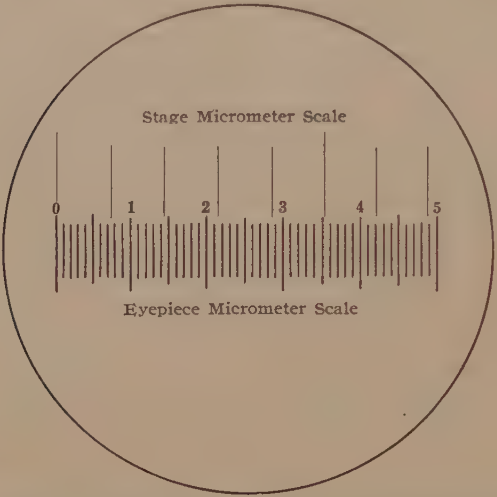

FIG. 141. Calibration of Eyepiece Micrometer. Scales not properly adjusted for comparison.

When high-power objectives are employed the rulings of the stage micrometer will appear as very thick or coarse lines. It then becomes essential to observe special precautions in the adjusting of the two scales, for if the adjustment shown in Fig. 143 *C* were to be followed, it is evident that an error would be introduced equal to at least half the thickness of the coarse rulings. Either the eyepiece micrometer scale rulings must be aligned with the centers of the coarser rulings of the stage micrometer, as shown in *A*, or at the right or left edges of them, but always all on the same sides, as shown in *B*. *By comparing as many divisions of each scale as possible,* any errors due to the width of the lines will be made more noticeable and may be corrected more perfectly. For instance, the central division of the stage micrometer in Fig. 143 *C* might at first glance be considered equivalent to five divisions of the eyepiece scale, but by comparing three or more of its divisions it is evident that this is not exactly the case but that its value is slightly less.

The value of the eyepiece micrometer divisions must be determined for each of the objectives used, and the draw-tube must in every case be adjusted to avoid the estimation of fractions and to give as simple ratios as possible. The values obtained should be recorded in tabular form (page 449), and all micrometric measurements in which they are used must be carried out with the particular tube length employed in the calibration for the respective objectives.

Construction of Micrometer Eyepieces. — Micrometer eyepieces should always be provided with a movable eye lens, in order that the scale may be focused as sharply as possible; the adjustment will vary considerably with the near- or far-sightedness of the user. If the eyepiece scale is to be projected, as in photomicrography, this adjustment is also useful in bringing the eye lens to such a position that the scale and the real image from the objective will both lie in the proper position to be projected simultaneously by it. Variation of the position of the eye lens does not alter the value of the scale divisions, since image and scale are affected equally.

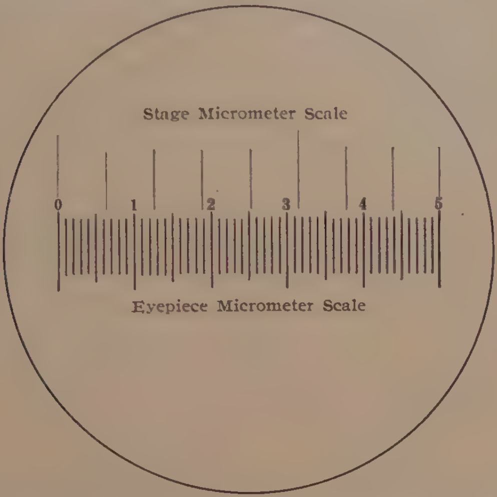

FIG. 142. Calibration of Eyepiece Micrometer. Scales properly adjusted.

Micrometer eyepieces are usually constructed so that the scale may be removed for cleaning, or different scales may be inserted in the plane of the diaphragm.

Micrometer eyepieces in which the entire scale may be displaced a short distance laterally by means of an ungraduated screw are of no particular value over those with fixed scales, especially if the microscope is equipped with a mechanical stage so that fine objects may be easily moved into position for measurement.

Negative or positive eyepieces are used for micrometry, but the latter are preferable for filar micrometers or other types in which movable slides are inserted in the focal plane.[13] The value of the divisions of the scale in a positive micrometer eyepiece is inversely proportional to the optical tube

[13] Wright: *Jour. Wash. Acad. Sci.* **1**, 60 (1911).

length, but the latter (not the reading on the draw-tube) must be known exactly if this is to be useful as an accurate means of varying the value of the scale.

Linear scales with simple numbered divisions are most useful for general work, but coördinate rulings are particularly convenient for counting particles or measuring areas (Figs. 144, 157, 158, and 159). Scales with various types of special rulings may be obtained from the different manufacturers.

In the ordinary micrometer eyepiece an eye and mental strain is often produced in counting the number of scale divisions, especially if the object is relatively large. To facilitate counting, Leitz has placed upon the market a scale, part black, part light, in which the divisions are sharply differentiated in blocks of ten, both horizontally and vertically (Fig. 145). This type of ruling has received the name of Step Micrometer, and is far less fatiguing than the older simple ruling.

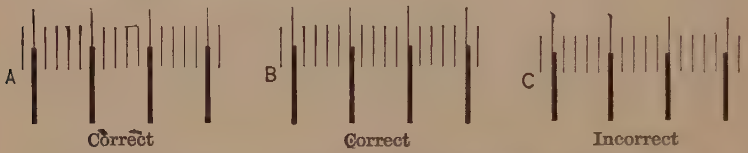

Fig. 143. Alignment of Rulings in the Calibration of an Eyepiece Micrometer.

For measurements under poor conditions of visibility the Gebhardt Contrast Micrometer (Fig. 146), made by Zeiss, will be found useful. In place of line rulings, which would be practically invisible, the scale consists of a row of tiny black squares touching at their corners. A scale of this type will stand out sharply, no matter how bright or dim the object may be.

Filar Micrometers. — In micrometry with eyepieces having fixed scales there is always the probability of error, since the magnitude of the real image as measured by the eyepiece scale usually requires an estimation of a fraction of a division. Very minute objects, even with high magnification, may fail to yield real images of sufficient size to fill a single division of the scale. To meet conditions such as these, filar micrometers are employed. In instruments of this kind, a scale or crosshair is made to traverse the field by means of a screw provided with micrometer thread, the amount of the movement being indicated by the revolution of a graduated drum attached to the screw head. Typical instruments of this class of micrometer eyepieces are shown in Figs. 147 and 148.

Before filar micrometers may be used for micrometry the value of one division of the ocular scale must be ascertained by means of a stage micrometer, according to the calibration procedure given above. The readings on the micrometer screw should also be compared with those of the ruled scale.

When using micrometers in which the diameter of the image of the object is measured by the movement of a micrometer screw, a number of observations should be made, always moving the crosshairs in the same direction to eliminate "back-lash."

To measure the length of an object by means of a micrometer eyepiece of the type shown in Fig. 147, first set the drum of the micrometer screw at 0, move the preparation until an edge of the image of the object is in contact with 0 on the scale. Count the number of whole divisions of the scale seen in the eyepiece; the fraction of a division that should be added to the number of whole divisions is ascertained by turning the micrometer screw so as to displace, to the right, the scale in the eyepiece until the end of the object just touches the scale division beyond which it originally extended. Read the drum, and add this fraction of a division to the reading first obtained.

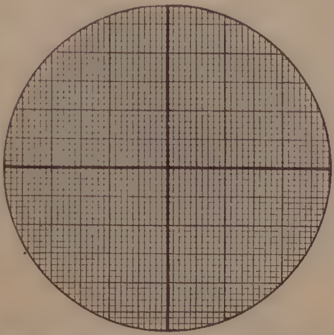

FIG. 144. Hutchinson Coördinate Ruled Eyepiece Micrometer. (James Swift & Son.)

Instruments of the type illustrated in Fig. 148 have a fixed scale within the eyepiece, across which travel crosshairs moved by a micrometer screw provided with a graduated drum. As in the type just described, one complete revolution of the drum is equivalent to one division of the scale within the eyepiece. The object to be measured is moved until the image falls under the scale and one edge in contact with one of the rulings. The number of whole divisions included within the image is recorded and the fraction of a division is ascertained by moving the crosshair and reading the drum.

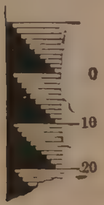

FIG. 145. Scale of Step Micrometer Eyepiece (Leitz).

For ordinary objects the first type described is more rapid but for very tiny objects such as pigments, etc., the second type is more convenient and somewhat more accurate.

Method 5: Projecting a Scale of Known Value into the Field of View by Means of a Substage Condenser.—This ingenious and practically universal method appears to have been first suggested by Goring about 1820, rediscovered by Pigott in 1870, and employed by Sorby in refractive index determination in 1878. It was again revived by A. E. Wright in 1890; thoroughly tested out by Ives in 1903, and independently rediscovered by Clendinnen in

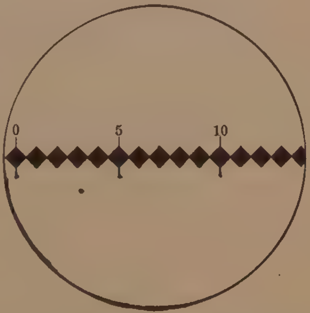

FIG. 146. Scale of Gebhardt Contrast Micrometer Eyepiece (Zeiss).

1910.[14] And yet in spite of the many times this principle of employing a scale of variable value as a standard has been independently discovered and its desirable features pointed out, it is almost never referred to in manuals devoted to microscopy.

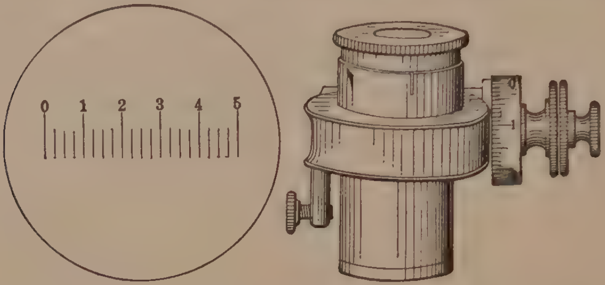

Fig. 147. Filar Micrometer Eyepiece with Movable Scale (Spencer Lens Co.).

By means of the mirror and the Abbe condenser, it is possible to project into the plane of the object lying upon the stage, the image of a scale whose value has been ascertained. Both scale and object are magnified together and it therefore follows that no matter what may be the combination of objective and ocular employed, the value of the divisions of the scale image will remain unchanged, provided that the distance of the scale from the condenser is not altered. Any change in the distance of scale from mirror and condenser will be accompanied by a proportional change

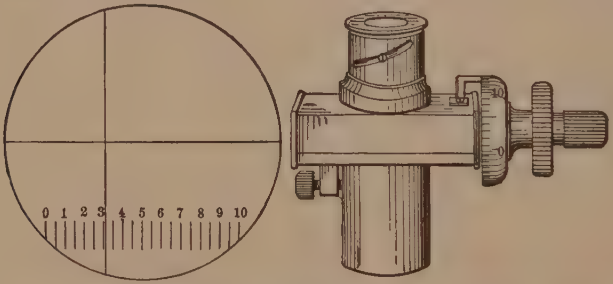

Fig. 148. Filar Micrometer Eyepiece with Movable Cross Hair (Bausch & Lomb).

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[14] See Ives: *Journ. Franklin Inst.*, **154**, 73; Clendinnen: *J. Roy. Micros. Soc.*, **1910**, 368.

in the size of the divisions of the scale in the image projected into the plane of the object.

In micrometry by means of ocular micrometers, we are restricted to the single ocular, containing the scale, and to a fixed draw-tube length. To obtain a different magnification, one is obliged to change objectives. This means that a new ocular micrometer value must be employed and a record kept for every change in objective. Moreover, the actual sizes of the divisions seen in the eyepiece micrometer are constant and cannot be changed.

In micrometry by means of a scale image projected by the condenser, we have merely to record the distance of the scale from the microscope in determining its value and we may then adopt any possible combination of objectives, oculars or tube lengths, without change of value.

A scale ruled as shown in Fig. 149 has been found satisfactory for general use. This scale, a photographic positive, is conveniently held in a vertical position by metal carriers attached to a cross-bar sliding upon a strip of wood 25 cm. long graduated in centimeters; this strip is attached to two small blocks, each the thickness of the base of the microscope stand. One block is notched at the end so as to permit its being always placed exactly in the same position (Ives' method). The best results are obtained when a strong source of artificial light is employed to illuminate the screen and a piece of ground glass is placed between the radiant and the scale-screen.

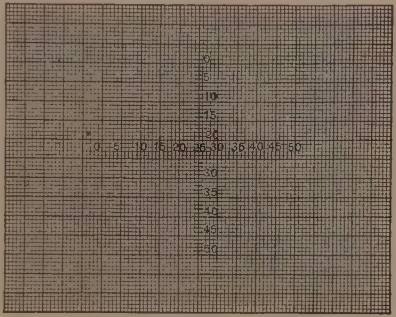

FIG. 149. Transparent Scale for Projection by Condenser in Micrometry.

The values of the scale are determined for three or more positions of the graduated strip and the results plotted upon coördinate paper. This is accomplished as follows: Place a stage micrometer upon the stage of the microscope, center and focus sharply using say a 16-mm. objective and 7.5 × eyepiece. Raise the substage condenser until the upper lens almost touches the object slide; open the iris diaphragm. Tip the *plane* mirror to one side and at the proper angle to throw an image of the scale into the condenser. Lower the condenser while looking into the microscope until the scale becomes clear and sharp. Turn the stage micrometer so that its graduations become parallel with those of the real image of the scale-screen. Move the stage micrometer until any line of the stage micrometer coincides with a line on the scale image. Count the number of division of the scale included in a division of the stage micrometer. Calculate the value for one division of the scale. Record the distance of the scale from the mirror as shown on the graduated strip and compute the value in microns as obtained for this position. Move the scale carrier to a new position and determine the value of a scale division as described above. In like manner find the true value for a third position. Plot the results upon a fairly large sheet of coördinate paper. This curve can then be employed in future measurements. It is obvious that the nearer the scale is to the microscope the greater will be the magnitude of the scale image, and the farther the scale the smaller the graduations will appear. Once the " curve " is obtained, we have at our command a device for accurate measurements (for all save very minute objects), by means of a scale the working magnitude of whose divisions is variable at will between wide limits.

This method of micrometry is especially convenient when employing binocular microscopes or where special rulings are required in quantitative work. The use of ruled glass cells is thus avoided.

Method 6: Measurements of Projected Real Images. — If the magnification of a real image projected by the microscope is known, any distance on that image may be measured directly with a scale, and its actual size computed.

Determination of the magnification of the projected image is an essential preliminary to such measurements. A stage micrometer is put in place of the object, and its divisions are projected on a screen under identical conditions of magnification. The divisions of the micrometer in the image are measured by a scale; their apparent size divided by their actual value gives the magnification. For example, if one division (0.1 mm.) measures 7.5 mm. in the image, the magnification is 75×.

By the converse of this procedure, the magnification may be adjusted to any desired value, by varying the factors which govern it until the proper ratio between the sizes of the scale divisions in the image and the stage micrometer is obtained (see page 255).

Measurements on the ground-glass of the camera or on a projection screen are particularly useful in case a great number of measurements have to be taken, since it is much easier to apply the scale than to move the specimen to the proper place on the micrometer each time. Where the character of the object permits an accurate rendition of its dimensions in a photomicrograph, the negative or positive may be projected on a screen by means of a lantern, and measured rapidly and conveniently at any time, without the need of keeping the specimen under the microscope during the entire process. In this case, the magnification of the photomicrograph and the magnifying power of the projection lantern must both be determined.[15]

Projected images of various small objects, such as screws, gears, machine parts, cutters, wire, and similar products, may be measured or compared against standards with great rapidity and ease.[16] Examinations of testing sieves for fineness and uniformity of wires and of openings may be carried out in this way.[17] A special microscope for measuring Brinell impressions and similar micrometry by projection has recently been placed on the market.[18]

Method 7: Measurements by Means of the Graduated Fine Adjustment. — If the microscope possesses a fine adjustment which is graduated, it may be used for measuring vertical distances. An objective of low penetrating power should be used, and the distance between the top and bottom of the object (or between " optical sections " at different levels) can be read on the micrometer screw.

The value of the divisions on the fine adjustment is either marked on the instrument or may be obtained from the catalog description. In the event that this information is unavailable, it may be obtained experimentally by placing upon a slide an object of known thickness and having plane parallel sides and clamping it tightly in contact by the stage clips. By focusing first on the slide and then on the top of the object, the number of divisions of the fine adjustment scale which is equivalent to the thickness may be measured. The actual thickness of the object may be determined by a dial gage, or by placing it edgewise and measuring it by any of the microscopic methods given above.

When employing the fine adjustment for micrometric measurements, all movements in focusing for readings should be made in the *same* direction, otherwise the lost motion and " lag " of the mechanism may introduce a serious error. It should also be borne in mind that few types of fine adjustments are so constructed as to have precisely the same value for one division throughout their entire range, and vertical measurements of 0.5 mm. or more may be somewhat inaccurate. To minimize this error as much as possible, especially when making comparative measurements, it is best to start all readings from the lower limit of the range of the movement.

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[15] This method, as applied to determinations of particle size of fine pigments, is fully described by Green: *Jour. Franklin Inst.* **192,** 637 (1921). See also:

Meade: *Ind. Eng. Chem.* **13,** 712 (1921).
Wightman, Trivelli, and Sheppard: *Jour. Phys. Chem.* **27,** 1 (1923).
Ramsden: *Jour. Oil Col. Chem. Ass.* **11,** 16 (1928).

[16] The Contour Measuring Projector of Bausch & Lomb has been developed for this purpose. See also Beck: *The Microscope,* Part II (1924), p. 160.

[17] Judson and Gould: *Jour. Optical Soc. Amer.* **6,** 719 (1922). A.S.T.M. *Standard Specifications for Sieves for Testing Purposes.* **E 11–26,** p. 3.

[18] Paul F. Hermann Co., Pittsburg.

Measurements of distances inclined to the axis of the microscope, such as the widths of slanting crystal faces, may be obtained from vertical and lateral measurements of the height h and horizontal projection p of the distance desired. The actual distance is then equal to $\sqrt{h^2 + p^2}$.

If a vertical measurement is made *within* a transparent object or mounting medium, it must be multiplied by the refractive index of the substance, to give the true distance;[19] the presence or absence of a cover glass having a different refractive index does not alter this requirement (see page 383).

Micrometric measurements by means of the fine adjustment are often called for in chemical work; for example, in ascertaining the depth of corrosion, checks or surface cracks, pits and elevations, and in actual measurements of the vertical dimensions of specimens.[20]

Applications of Linear Measurements. — Nearly all microscopic measurements are ultimately linear in character and there is no field of microscopical work where quantitative determinations of dimensions are not important. Specific discussion of all the various applications of micrometry cannot be given here, but the adaptability of the methods outlined above should be readily apparent. The use of linear measurements as a basis for ascertaining area and volume is given below.

"PARTICLE-SIZE" DETERMINATIONS

Within comparatively recent years it has been realized that the degree of fineness of powdered materials is of the utmost importance, for whatever purpose the material may be used, and that the ordinary methods of size-analysis by standard sieves are inadequate for elongated, flaky, or very fine particles.[21]

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[19] Addey: *Jour. Queckett Micros. Club* (2) **14**, 279 (1922).
Metzner: *op. cit.*, p. 170.

[20] The measurement of varnish films by means of the fine adjustment is recommended by Stoppel: *Proc. A.S.T.M.* **23**, I, 285 (1923).

The volume or surface of a particle which just passes through a sieve opening of a given size may be estimated fairly accurately, if the particle is roughly equidimensional (spherical or cubical) in shape. But if the material has a tendency to yield platy or elongated particles when pulverized, the sieve opening through which these pass will not give direct information as to all their various dimensions, or their volume or surface.

In formulating specifications based on sieve tests of powdered materials, the actual desired volume or surface of the particles should be kept in mind, if this is of direct importance. Specifications may need to be revised in the light of microscopic study of the particles which are passed or retained by the testing sieves, so as to select material of the proper dimensions. Certain minerals with well defined cleavage yield elongated particles which behave in screen tests as if they actually contained much less material (Fig. 152). Flaky particles may fail to go through a sieve even when their volume is much smaller than that of spherical particles which pass easily. Control of pulverizing operations is not complete without information as to the shape of the particles which the raw material yields under the grinding methods which are employed; and for this information microscopic examinations are essential. Microscopic control is also useful in connection with the grinding of materials which, although not excessively fine, cannot well be graded by screening. Abrasive powders, which would rapidly cut the wires of the sieve, or substances which might cake, may be examined microscopically, and the process regulated to give the proper particle-size.

Testing sieves afford merely a means of measuring the powders which *fail* to pass through them. For particles which are not retained, finer sieves must be used. Any ground material and most powders prepared by precipitation contain a large proportion of particles smaller than the limit of fineness of sieves (Figs. 150 and 151).

It is these fine particles, which will pass through a "325 mesh" sieve (opening 44 μ),[22] that have the greatest significance in many pulverized materials, and nearly all important pigments and fillers come within this range. For the finer pigments and fillers, screen tests indicate nothing more than the presence or absence of coarse aggregates, since the actual diameter of the largest single particles may be less than one-fiftieth that of the screen opening which is supposed to grade the material.

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[21] Ladoo: *Chem. Met. Eng.* **30**, 623 (1924).
Kanowitz: *idem.* **32**, 199 (1925).

The manifold importance of particle-size in governing the properties of finely divided material is too extensive for discussion in detail here. In many instances substances are prepared in pulverized form simply to reduce their dimensions for mechanical purposes, as in the case of abrasive powders or coarse fillers in paints and rubber. Where really fine particles have been found necessary, their advantages are largely due to the greatly increased surface for a given volume (or weight) of substance. The surface presented by fine pigments is relatively enormous (roughly 2 square meters per gram) and a decrease in particle-size increases this proportionately.

Microscopic study affords the most positive method of testing finely divided material which is beyond the range of standard sieves. Not only is the size of the particles measured, but shape, uniformity, aggregation, and other important properties which affect the ultimate usefulness of the material, are observed directly. Various non-microscopical methods are available for particle-size determinations but they depend for confirmation upon microscopic studies, and should never be used to the exclusion of direct examinations.

Microscopic particle-size determinations have been found significant in studying phenomena of which the following are typical examples:

The setting and strength of cement,[23] the density and strength of porcelains[24] and other ceramic products, the activity of insecticides[25] and of drugs, the stability of emulsions,[26] the settling and collection of dusts,[27] the pulverization of coal[28] the mechanism of filtration,[29] the grinding of fine powders,[30] the annealing and hardness of brass[31] and other metals, fat globules in milk,[32] the extent of milling required for separating ore minerals,[33] the length of paper fibers[34] pulped by different processes, and the fineness of paper fibers,[35] the covering power,[36] opacity,[37] yield value[38] and other properties of paints[39] governed by their pigments, the sensitivity of photographic emulsions[40]

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[22] A.S.T.M. *Specification* **E 11–26** recommends the designation of sieves by means of the diameter of their openings in microns, rather than by the number of " meshes per inch," which may vary with the diameter of the wires.

[23] N. C. Johnson: *Thesis, Cornell University,* **1913**

Hauenschild: *Tonind. Ztg.* **38**, 477; *Zement* **15**, 453, 469, 488 (1926).

Guttmann: *Zement* **15**, 164, 185, 200 (1926).

[24] Walker: *Trans. Amer. Ceram. Soc.* **18**, 499 (1916).

Craven: *Jour. Soc. Chem. Ind.* **41**, 329T (1922).

Arnold: *Jour. Amer. Ceram. Soc.* **6**, 409 (1923).

Geller: *Ceramist* **6**, 708 (1925).

[25] Griffin *et al*: *Jour. Agr. Res.* **34**, 727 (1927).

[26] Finkle, Draper and Hillebrand: *Jour. Amer. Chem. Soc.* **45**, 2780 (1923).
Krantz and Gordon: *Jour. Amer. Pharm. Ass.* **15**, 83 (1926).

[27] Theissen: *Bur. Mines Bull.* **82**, p. 56.
Lanza and Higgins: *Bur. Mines. Tech. Paper* **105**, p. 22 (1915).
Higgins, Lanza, Laney and Rice: *Bur. Mines Bull.* **132**, p. 22 (1917).
Drinker, Thompson and Fitchet: *Jour. Ind. Hyg.* **5**, 162 (1923).
Trostel and Frevert: *Ind. Eng. Chem.* **15**, 232 (1923).

[28] Kreulen: *Brennstoff-Chemie* **5**, 28 (1924).
Bouton and Pratt: *Carnegie Inst. Coal Min. Invest. Bull.* **12** (1924).
Andrews: *Mech. Eng.* **47**, 429 (1925).

[29] Hickson, Work, and Odell: *Trans. Amer. Inst. Min. Met. Eng.* **73**, 225 (1926).

[30] British Portland Cement Research Association: Researches on the Theory of Fine Grinding.
Martin: *Jour. Oil Col. Chem. Ass.* **9**, 244 (1926); *Trans. Inst. Rubber Ind.* **2**, 125 (1926); *Trans. Ceram. Soc. Eng.* **25**, 21, 226, 240, etc. (1926), **27**, 247, 259, 285 (1928).
Andreasen: *Kolloidchem. Beihefte* **27**, 349–458 (1928).

[31] Bassett and Davis: *Trans. Amer. Inst. Min. Met. Eng.* **60**, 428 (1919).

[32] Cooper, Nuttall and Fink: *Jour. Agr. Sci.* **5** (3) June, 1913.
Williams: *Jour. Roy. Micros. Soc.* **273**, 418 (1925).
Beckett: *Econ. Proc. Roy. Dublin Soc.* **2**, 303 (1927).

[33] Hynes: *Min. Sci. Press* **110**, 994 (1905).
Campbell: *Eng. Min. Jour.* **103**, 929 (1917).
Clayton: *Chem. Met. Eng.* **19**, 61 (1918).
Thomson: *Amer. Mineral.* **8**, 99 (1923).

[34] Graff and Hogdon: *Paper* **23**, 11 (1918).

[35] Lorenz: *Papierfabr.* **23**, 753 (1925), **24**, 33, 74, 91 (1926).

[36] Kuehn: *Farben Ztg.* **31**, 1131 (1926).

[37] Spear and Endres: *Ind. Eng. Chem.* **15**, 725 (1923).
Rhodes and Fonda: *idem.* **18**, 130 (1926).

[38] Green and Haslam: *Ind. Eng. Chem.* **19**, 53 (1927).

[39] Klein and Hulme: *Jour. Oil Col. Chem. Ass.* **3**, 117 (1920).
Klein and Parrish: *idem.* **7**, 54, 82 (1924).
Parrish: *idem.* **8**, 195 (1925), **9**, 252 (1926).
Klein: *idem.* **9**, 192 (1926).
Green: *Chem. Met. Eng.* **28**, 53 (1923).
Audubert and Rabaté: *Chimie et Ind.* Special No., Sept. **1925**, p. 486.

[40] Wightman, Trivelli, and Sheppard: *Jour. Phys. Chem.* **25**, 181, 561 (1921); **27**, 1, 141, 466 (1923); **28**, 529 (1924).

the reinforcing value of fillers in rubber,[41] and the chemical reactivity of various finely divided solids such as zinc dust in the cyanide process,[42] or lead oxides in storage batteries.[43] The dynamic chemical properties of nearly all heterogeneous systems are dependent upon surface effects, and therefore upon particle-size.

Experimental Factors Affecting the Value of Particle-Size Determinations. — The character of the microscopic image, as determined by the aberrations and resolving power of the instrument, the proper adjustment of the illumination, the perfection of focus, and the sharpness of the outline of the particles in the mounting medium used, limits definitely the precision with which measurements can be made. Satisfactory results may be unobtainable unless each of these factors is at its optimum, and gross errors may be introduced if observations are made without careful attention to all of them. The accuracy of calibration will not involve the possibility of significant error, provided no mistakes of mathematics have been made.

The shape of the particles to be measured introduces a serious complication, since it is hardly practicable to determine all the dimensions of each of a large number of tiny particles. If shape is not taken into account, however, there is little point in making elaborate statistical measurements, since these may have almost no significance in the ultimate application of the material.[44] In general it may be stated that irregular particles may be classified by estimation in terms of an equivalent sphere or cube, without introducing a serious error. Markedly elongated or flattened particles may be measured in two directions, and the third dimension estimated, the " diameter " being calculated either as that of a cube of equivalent surface or volume or as the harmonic mean of the three dimensions.

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[41] Wiegand: *Can. Chem. Jour.* **4**, 160 (1920).
Shippel: *Ind. Eng. Chem.* **12**, 33 (1920).
Green: *idem.* **13**, 1029 (1921).
Endres: *idem.* **16**, 1148 (1924).
Twiss: *Jour. Oil Col. Chem. Ass.* **9**, 197 (1926).
Pickles: *idem.* **9**, 204 (1926).
Klein: *Trans. Ind. Rub. Ind.* **2**, 73 (1926).
Twiss: *idem.* **2**, 78 (1926).
Heaton: *idem.* **2**, 96 (1926).

[42] Lepsoe: *Trans. Amer. Inst. Min. Met. Eng.* **1407–B.** Feb. 1925.

[43] Reinhardt: *Ind. Eng. Chem.* **19**, 1124 (1927).

[44] The influence of the shape of particles on their usefulness is discussed by:
Vogt and Evans: *Ind. Eng. Chem.* **15**, 1015 (1923).
Schidrowitz: *Jour. Oil Col. Chem. Ass.* **9**, 208 (1926).

$$\frac{3\, l \cdot b \cdot t}{lb + lt + bt}.$$

For rapid estimations, reasonably accurate results may be obtained by considering the average of the three dimensions of a particle, or the lesser of its two horizontal dimensions, as its diameter. This may be accomplished by inspection, as each particle is counted.[45]

The size, uniformity, and degree of aggregation of the particles affect the accuracy with which they can be measured individually. Single particles less than 0.5 μ in diameter cannot be measured exactly, and their size can be estimated only by averaging the measurements of a number of them. If very large and very small particles occur in a sample, they cannot be focused simultaneously, and photographic methods of measurement are ruled out unless the material is first separated into different size-groups.

If the sample, as received, contains aggregates of particles, these may be broken up for measurement of the grains which compose them. There is a slight risk of subdividing the individual particles, but in most methods of preparation the dispersion is accomplished largely by shearing forces in the suspending medium rather than by true crushing or grinding action. However, there is no assurance that the degree of separation of the particles in the microscopic preparation corresponds to that which will actually be effected when they are used; for instance, as milled into paint or rubber. For this reason it is advisable to make particle-size determinations on the final product, if possible, or at least to compare the dispersion of the particles in it with that on the microscope slide.

Computations from Particle-Size Data. — In addition to the above sources of "experimental error," improper analysis of results must be guarded against. The measurements of the individual particles may be unquestionably accurate, yet an attempt to summarize these statistically may be entirely misleading. Any effort to express the dimensional properties of a powdered material by means of one or two numerical terms is inadequate at best, and should not be considered as a substitute for any further microscopical examinations. The term " average particle-size " is almost meaningless unless the basis of its determination is definitely specified.

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[45] Detailed treatments of the relationship of particle-shape to particle-size determinations are given by:

Ashley and Emley: *Ind. Eng. Chem.* **3**, 87 (1911).
Martin, Blyte and Tongue: *Jour. Ceram. Soc.* **23**, 61 (1924).
Wolff: *Farben Ztg.* **32**, 1667 (1927).
Green: *Jour. Franklin Inst.* **204**, 713 (1927).

Before undertaking any quantitative determinations of particle-size, other than those based on approximate comparisons with standard samples, a thorough study of the methods of expressing results should be made, and the distinction between them should be clearly understood. The methods, calculations and examples given in connection with the fundamental investigations by Green,[46] Wightman, Trivelli, and Sheppard,[47] Perrott and Kinney,[48] Weigel,[49] and Work[50] should be followed through in detail.[51]

Although it is possible to estimate by inspection the " average diameter " of a mixture of fine particles, this is of little value except for purposes of rough comparison, and may often be deceptive, especially if the sample contains particles of a wide range of sizes. The best means of studying the particle-size of a material is by determination of the numbers of particles which fall within fairly narrow size-groups throughout the entire range of sizes. The " frequency " of each size may be tabulated, or plotted in a **" size-frequency curve,"** and these data serve as an adequate basis for all necessary computations of averages and of specific surface.

Since the number of particles of different sizes is not of direct practical significance, the data may be recalculated to give the portion of the total surface or volume (weight) included in each of size-group:

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[46] *Jour. Franklin Institute* **192**, 637 (1921); **204**, 713 (1927); *Jour. Ind. Hyg.* **7**, 155 (1925); *Chem. Met. Eng.* **28**, 53 (1923).

[47] *Jour. Phys. Chem.* **25**, 181, 561 (1921); **27**, 1, 141, 466 (1923); **28**, 529 (1924).

[48] *Jour. Amer. Ceram. Soc.* **6**, 417 (1923).

[49] *Bur. Mines. Tech. Paper.* **296** (1924).

[50] *Proc. A.S.T.M.* **28** II. 771–812 (1928).

[51] An exhaustive mathematical analysis of the various methods of calculation is given by Loveland and Trivelli: *Jour. Franklin Inst.* **204**, 193, 377 (1927). See also Hatch and Choate: *idem.* **207**, 369–87 (1929).

$$\text{Fraction of total surface} = \frac{nd^2}{\Sigma nd^2}$$

$$\text{Fraction of total weight} = \frac{nd^3}{\Sigma nd^3}$$

These calculated values may be used to plot a "surface" or "weight distribution curve" which will show at a glance the percentage of the total surface or weight of the material which is due to particles of different sizes.[52]

The various curves referred to above are particularly valuable since they indicate the uniformity of the material, a property which is concealed if the "average particle-size" is used as a substitute.

The simplest method of averaging is based on the "size-frequency curve." It consists of totaling the products of frequency n by diameter d, and dividing this by the total number of particles studied. This gives the "numerical average particle diameter,"

$$\frac{\Sigma nd}{\Sigma n}.$$

Such an average is easily calculated, but has little actual physical meaning, and is useful principally in describing materials of markedly uniform particle-size. It corresponds approximately to the position of the maximum of the size-frequency curve, if a well-defined maximum exists.[53]

The averages based on "surface" or "weight distribution curves" are distinctly more significant, especially in the case of powdered materials having a wide size range. They are computed in an analogous manner:[54]

$$\text{Surface average particle diameter} = \frac{\Sigma nd^3}{\Sigma nd^2}$$

$$\text{Weight average particle diameter}^{55} = \frac{\Sigma nd^4}{\Sigma nd^3}$$

[52] A number of these different types of curves are shown in the papers by Weigel, and by Perrott and Kinney.

[53] Averages of this type were used by Green (*loc. cit.*) in the study of fine pigments, and by Wightman, Trivelli and Sheppard (*loc. cit.*) in the study of silver bromide grains in photographic emulsions.

[54] Averages of this type are recommended by Perrott and Kinney (*loc. cit.*) and by Weigel (*loc. cit.*), in the study of powdered coal, and of ground pigments and fillers.

[55] This corresponds to the "mass mean radius" of Nichols and Liebe: *Third Colloid Symposium Monograph* (1925) p. 283.

These averages may be interpreted physically as the diameters above and below which there are equal surfaces (or weights) of material, and they correspond approximately to the maxima of their respective surface or weight distribution curves.

The distinctions between the different methods of averaging have a very real importance, as indicated by examples from actual specimens:

Average Diameter	*Powdered Coal*[56]	*Ground Ochre*[57]
based on number	$3.0\ \mu$	$0.67\ \mu$
surface	$21.0\ \mu$	$4.77\ \mu$
volume	$36.4\ \mu$	$14.72\ \mu$
number of particles $< 2\ \mu$	82%	*circa* 97%
weight of material $< 2\ \mu$	1.5%	*circa* 1%

These discrepancies become less, the more uniform the particle-size. Since surface properties are usually most directly related to the usefulness of the material, the " surface average particle diameter " is likely to be most significant. As pointed out by Green,[58] a comparison of two or more substances on the basis of one type of average diameter can give no direct indication of their comparative properties with respect to an average diameter calculated on a different basis.

Average particle-size, as calculated by any of the above methods, does not indicate the uniformity of the material. This may be expressed fairly well by the " uniformity coefficient,"[59]

$$\sqrt{\frac{n}{2\ \Sigma v^2}}$$

(where v is the variation of each size-group from the average).

" Specific surface " is a numerical measure of the surface, in square meters, presented by one gram of the material. It is equal to

$$\frac{6}{\text{density} \times \text{average size}}.$$

The " average size " used should be based on surface, unless the material is highly uniform.

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[56] Perrott and Kinney: *op. cit.*, p. 421.

[57] Weigel: *op. cit.*, p. 24.

[58] *Jour. Franklin Inst.* **204,** 719 (1927).

[59] This must not be confused with " coefficient of uniformity " as used in the size-analysis of filter sands.

All the quantitative methods of determining particle-size involve rather time-consuming statistical studies, and various means have been employed in the investigations cited, to simplify the time required for the numerous measurements and calculations. Projection methods, counting tallies, adding and multiplying machines, tables of squares and cubes, and similar aids are useful if such work is to be done.

Methods of Determining Particle-Size. — **Estimations**, based on inspection, are likely to be in error, unless the material is relatively uniform. If the proportion of the material included in each of several different size-groups is estimated, the results will have more value. Comparisons with standards, of known satisfactory fineness, may be adequate for ordinary testing and control purposes. A comparison microscope should be used, and the samples should be dispersed and mounted as nearly identically as possible. Comparisons are most valuable in the case of angular or splintery particles, and are most misleading in the case of materials which are distinctly non-uniform in size.

Preliminary examination of the sample is always worth-while, as it throws light on its size, uniformity, shape, visibility, and ease of dispersion, all of which will govern the choice of procedure for quantitative studies.[60]

Method 1: Zsigmondy's Method. — The principles employed in the determination of the size of ultramicroscopic particles are applicable to coarser material also.[61] The method is essentially a determination of the *number* of particles in a given weight of powdered material. The quantity of material examined is controlled by making up a suspension of known concentration, and counting the particles in an aliquot portion of this.

The volume of the suspension counted may be controlled by either of two methods:

(*a*) A layer of known thickness is prepared, and a measured area of this is counted. For such films plane slides and cover-glasses are necessary, and cells such as those used for blood counts (haemacytometer cells) or mold counts, holding a definite depth of liquid, are preferable.[62] The cells furnished with the cardioid ultramicroscope may also be satisfactory, if their depth is accurately determined by means of the graduated fine adjustment of the microscope.

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[60] Methods of preparation and mounting are given on p. 169, and in the references already cited.

[61] A discussion of the principles and limitations of this method has been given in Chapter VII.

[62] Cells of various types are described on pp. 435–436.

The counting is done with an appropriate objective, usually under dark field illumination. Dilution, or the counting of small fractions of the field, may be necessary in the case of concentrated suspensions. If Brownian movement is troublesome, a suspending medium which is very viscous, or which sets to a gel, may be used, or the particles may be allowed to settle on the cell surfaces before counting.[63] Very careful cleaning of the cell is necessary, and checks with bright field illumination are desirable, in order to eliminate errors due to foreign particles.[64]

(*b*) The particles illuminated by the beam of the slit ultramicroscope are counted, the volume of suspension being determined by an eyepiece micrometer (page 233). This procedure is hardly suitable for any but ultramicroscopic particles.

In the volumes of suspension defined by either of the above methods, a sufficient number of counts should be made to give satisfactory checks with different fields and different preparations of the same sample. The method of calculating the size of the particles is given on page 234.

Zsigmondy's method of counting ignores any lack of uniformity in the size of the particles, and is best suited to materials which are naturally very uniform, to sized fractions of less uniform material, or to particles too fine to be measured accurately by other methods. Well dispersed gas blacks or very fine zinc oxides may well be measured by this procedure.

Method 2: Green's Method.[65] — The number of particles of each different size is counted, tabulated, and plotted as a " size-frequency curve." From the tabulation or the curve the " numerical average particle size " and the " uniformity coefficient " may be estimated by inspection, or computed according to the methods given on page 415.

Dry-mounted preparations, prepared by the method given on page 170, are preferable on account of their greater visibility and absence of Brownian movement, and because the particles, if fairly uniform, are all in focus at once. If the resolving power of dry objectives is insufficient, immersion objectives may be used on such preparations, the particles being mounted in a medium of appropriate refractive index and covered with a cover-glass.

The actual measurement may be made directly by means of an eyepiece micrometer, or preferably by a photographic method. The latter involves obtaining a perfect negative, in which all the particles are sharply focused and clearly resolved; if the outlines of all the particles do not appear perfectly sharp, and their centers transparent, the value of measurements based on such images is questionable. The photograph is measured as enlarged upon the screen by a projection lantern. Besides a certain saving of labor in routine work, the photomicrographic method has the advantage of supplying a permanent record of the actual appearance of the sample, which may present features hardly to be summarized in the numerical results of the calculations based on it.

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[63] Kühn: *Zeits. angew. Chem.* **28**, 126 (1915); *Farben Ztg.* **31**, 1131 (1926).

[64] Wightman and Sheppard: *Jour. Phys. Chem.* **25**, 571, 580 (1921).

[65] *Jour. Franklin Inst.* **192**, 637–66 (1921).

A critical discussion of this method is given by Hebler in Liesegang's *Handbuch der Kolloidchemische Technologie* (Leipzig, 1926), p. 170.

A total of two hundred or more particles, occupying one or two representative fields, should be measured; if the distribution is uneven, or the uniformity is low, more measurements may be advisable.

Green's method is appropriately applied to material which is of microscopic dimensions, of good visibility, and relatively uniform in particle-size (Fig. 151), as exemplified by precipitated or "fume" pigments and fillers.[66]

Method 3: Perrott and Kinney's Method.[67] — Every particle in a given portion of the sample is counted, and its size estimated. A "surface" or "weight distribution curve" is prepared, or equivalent data are tabulated, and the "surface" or "weight average particle diameter" is computed by the methods given on page 415.

Dry-mounted preparations are generally preferable, on account of their good visibility, but mounting in a viscous liquid may be necessary to insure uniform distribution of particles of various sizes. Particular care should be taken to avoid partial segregation of the material into fine and coarse fractions during the process of preparing the slide.

Photographs are useful for purposes of record, but visual measurement with an eyepiece micrometer is usually necessary, on account of the difficulty of focusing all particles sharply if the uniformity is low. The finer particles need be counted and measured in only a few fields, since they are present in large numbers, and are likely to be well distributed. The larger particles in many fields must be measured, on account of their low frequency and the risk of segregating them in the preparation. All measurements and frequencies are finally computed in terms of the same amount of sample, usually that contained in one field.

[66] Fine emulsions have been measured both by Zsigmondy's and Green's methods, by van der Meulen and Rieman: *Jour. Amer. Chem. Soc.* **46**, 876 (1924).

Starch grains have been measured by Green's method, by Lindet and Nottin: *Ann. fals.* **16**, 134 (1923).

[67] *Loc. cit.*

Weigel: *op. cit.*, pp. 6–18.

A finely divided coördinate eyepiece micrometer such as that shown in Fig. 144, is particularly useful. The markings serve to divide the field and also permit measurements to be made in any part of it without the need of moving the preparation.

Perrott and Kinney's method is applicable to all types of powdered material, but is particularly useful in dealing with samples consisting entirely of resolvable particles and exhibiting a wide range of sizes, as exemplified by ground pigments and fillers (Fig. 150).

Method 4: Fractionation Methods. — Material which is highly non-uniform in particle-size is separated into fractions which are more uniform, and these are measured and calculated separately, the data from them being combined in the final results. The fractionation may be accomplished by sedimentation in water, or in viscous liquids such as glycerin,[68] gelatin,[69] or celluloid[70] solutions, by centrifuging,[71] or by elutriation in a rising current of liquid.[72]

The mounting and measurement of each fraction may be carried out by Method 2 or by Method 3, depending on the uniformity of the particles which it contains. If Method 3 is used, the chief advantage of fractionation over direct measurement and counting of the total sample is in convenience and accuracy of distribution and measurement of the preparation.

Non-microscopical methods of determining particle-size and frequency are usually time-consuming and require large amounts of material. They are ordinarily no more accurate than microscopical methods, but do not necessarily require such skilled technique. In most cases their results are checked by microscopic studies. Extensive bibliographies of the various methods and their applications are given by Klein and Parrish.[73]

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[68] Wightman and Sheppard: *Jour. Phys. Chem.* **25**, 561 (1921).

[69] Renwick and Sease: *Second Colloid Symposium Monograph* (**1924**), p. 37.

[70] Baltrusch: *Zeits. angew. Chem.* **40**, 1450 (1927).

[71] Wightman and Sheppard: *loc. cit.*

[72] Weigel: *op. cit.*
Lowry: *Trans. Faraday Soc.* **18**, 23 (1922).
Klein: *Oil Col. Tr. Jour.* **69**, 790 (1926).
Shafor: *Ind. Eng. Chem.* **19**, 573 (1927).

[73] *Jour. Oil. Col. Chem. Ass.* **3**, 177 (1920); **7**, 54, 82 (1924); **8**, 195 (1925); **9**, 252 (1926). See also Report of Section on Sub-Sieve Sizes. *Proc. A.S.T.M.* **28**, I, 640 (1928); and Gonnell: *Zement* **17**, 1786, 1819, 1848 (1928).

MEASUREMENTS OF AREA AND VOLUME

Measurements of area are important in connection with studies of particle-size, and various other quantitative microscopical investigations, particularly in the areal analysis of heterogeneous mixtures (page 443).

The area of simple geometrical shapes, such as the faces of crystals or the cross-sections of certain cylindrical fibers, may be determined by calculation from linear measurements of their dimensions. Areas of irregular shape can be rapidly estimated in terms of equivalent squares or circles, by using an eye-piece micrometer with concentric square or circular engravings, or by analogous methods on drawings or photomicrographs.

More exact areal measurements may be made by means of a coördinate-ruled eyepiece micrometer, the number of squares included in the area being counted.[74] Tracings may be made on coördinate paper by means of a drawing camera, or a transparent ruled scale may be superposed upon a photomicrograph, the squares being counted similarly.

A planimeter may be used to measure the area of projected images, or of tracings or photomicrographs. A simpler method is to cut out the area from the drawing or picture, by means of fine scissors, and to weigh it on a balance; tracings on tinfoil may be used if desired. From the weight per unit area of the paper or tinfoil, the area of the cut-out portion may be calculated with considerable accuracy.[75]

The determination of grain-size in metals is essentially a determination of the number of grains intersected by a surface of unit area, 1 sq. mm.[76] The image of the polished and etched specimen is projected on a plate of ground-glass such as the focusing screen of a metallograph. The number of grains in an area of 5000 sq. mm. on the image is counted. This area may be marked on the smooth side of the screen as a circle of 79.8 mm. diameter or a square of 70.7 mm. edge. Half the grains intersected by the edge of the marking are counted with those included in the area. The grains may be checked off by means of a glass-marking pencil. The number of grains counted is multiplied by a factor depending on the magnification, $\frac{\text{mag.}^2}{5000}$, to give the number per square millimeter of surface on the specimen. If instead of expressing the grain-size as the number of grains per square millimeter, it is desired to express it in terms of the diameter of the average grain, the reciprocal of the square root of this number is taken. If the area of the average grain is to be expressed in square microns, this may be obtained by multiplying the reciprocal of the number by 1,000,000. The actual physical significance of these " averages " is questionable, unless the uniformity of the grains is fairly high.

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[74] This method is used in measuring the cross-section of rayons, by A. Herzog: *Mikroskopische Untersuchung der Seide und Kunstseide* (Berlin, 1924), p. 28.

[75] Delessé: *Comptes rendus* **25**, 544 (1847); *Ann. des Mines* **13**, 379 (1848).

Johannsen: *Manual of Petrographic Methods* (1918), p. 290.

This method was used by Stamm: *Fourth Colloid Symposium Monograph* (1926), p. 246, to measure the proportion of the voids in the cross-section of wood.

[76] Jeffries, Kline and Zimmer: *Trans. Amer. Inst. Min. Met. Eng.* **54**, 594 (1916).

Jeffries: *Chem. Met. Eng.* **16**, 503 (1917); **18**, 185 (1918).

A.S.T.M.: *Standard Rules for the Preparation of Micrographs*, **E2–24**.

Grain-size determinations and specifications are becoming widely recognized as a means of controlling the annealing, hardness, and ductility of alloys, particularly brasses.[77] Similar determinations have been found useful in the study of refractories and of stone for construction.

Measurements of volume are of wide usefulness in the estimation of weight of microscopic objects, and in quantitative analyses of heterogeneous mixtures of powdered materials.

If the object under investigation is approximately equidimensional, its volume may be estimated by inspection in terms of an equivalent sphere or cube. This is generally accurate enough for use in quantitative analyses of mixtures where the number and size of various kinds of grains must be taken into account. If the grains are markedly elongated, actual measurements of each one may be necessary, since estimations are inaccurate on such material.

The volume of an object of simple geometrical form, such as a sphere or a crystal, may be computed from careful measurements of its dimensions. The specific gravity can then be calculated if the weight is known, or vice versa (page 199).

Measurements of volume may be applied directly to a number of types of chemical problems. The determination of weight of very tiny particles is relatively simple, even though they may be difficult to separate from surrounding material, and beyond the range of the ordinary micro-balance. If the substance can be made to take the form of a sphere, the results may be highly accurate, and even with irregular particles very useful estimations may be made, which are unobtainable by other means.

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[77] The relationships existing between the heat treatment, grain size, and mechanical properties of brasses of different compositions are well brought out by Bassett and Davis: *Amer. Inst. Min. Met. Eng. Trans.* **60,** 428 (1919); *Techn. Pub.* **E–26** (1927).

The use of microscopic measurements as a substitute for weighing in assaying is very old, and has been shown[78] to be accurate as well as rapid, if carefully fused spherical beads of metal are prepared. Blowpipe assaying may be carried out on small samples, since only a tiny " button " is necessary for measurement. The gold, silver, or platinum obtained may be fused on charcoal or in a borax bead, transferred to a slide, and measured. The outline of the bead should be sharply focused in silhouette against a moderately bright field; several different diameters should be determined, and the results averaged. The weight may then be calculated from the formula:[79]

$$\textit{weight} = (\textit{diam.})^3 \times 0.5236 \times \textit{specific gravity}$$

This method was employed by Miethe and Stammreich[80] in their investigations of the minute amounts of gold contained in mercury.

In toxicology[81] and in the analysis of urine, mineral waters, gases and organic compounds containing mercury, microscopic measurements constitute one of the oldest and best methods of determining the minute quantities which are usually encountered. Raaschou[82] has studied in detail the methods for quantitatively separating amounts of mercury from liquids. When dealing with mercury condensed as tiny globules[83] after heating the copper on which it has deposited from solution, the tiny spheres may be made to coalesce to a few large ones, by stirring the deposit with a fine needle or glass rod, or a stiff hair. In order that accurate measurements may be made, the spheres should not be so large as to flatten appreciably. Booth, Schreiber, and Zwick[84] avoid this difficulty in the case of relatively large amounts of mercury by placing it in a measured capillary, and determining the length of the column — in other words, the volume of a cylinder instead of a sphere.

Bubbles of gas may also be measured with considerable accuracy under the microscope, since they are spherical if not too large. Krogh[85] carries out micro gas analysis by measuring the initial diameter of the bubble, drawing it into a tiny pipette containing an absorbing reagent, expelling, and measuring again. Emich[86] has estimated the weight of ultramicroscopic particles of zinc by measuring the diameter of the bubbles of hydrogen liberated by them from acid. Baylis[87] used a succession of drawings, made by means of a drawing camera, to follow the growth of large bubbles at the expense of small ones, in investigating the causes of air-binding of filters.

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[78] Goldschmidt: *Zeits. anal. Chem.* **16**, 434 (1877).
Lunde: *Mikrochemie* **5**, 16, 102 (1927).

[79] The diameter must be expressed in centimeters. The specific gravities of some metals for which this method is used are: gold — 19.33; silver — 10.4; platinum — 21.15; lead — 11.36; mercury — 13.59. Calculated weights are usually accurate to less than 0.01 mg. (10 μg. or 10 γ).

[80] *Zeits. anorg. Chem.* **140**, 368 (1924).

[81] Blyth: *Poisons; Their Effects and Detection* (London, 1884), p. 248.

[82] *Zeits. anal. Chem.* **49**, 172 (1910).
See also Hartung: *Jour. Inst. Metals* **33**, 427.
Bodnar and Szep: *Biochem. Zeits.* **205**, 219 (1929).

[83] See also Vol. II. *Detection of Mercury.*

[84] *Jour. Amer. Chem. Soc.* **48**, 1815 (1926).

Measurements of volume by means of micro-pipettes are discussed in Vol. II. Other methods of measuring volume are described in connection with their application in the analyses of heterogeneous mixtures, Chapter XIII.

The thickness of protective coatings such as paint, varnish, lacquer, enamels, glazes, platings, etc., is studied under the microscope, particularly in connection with investigations on their resistance, opacity, and the amount of coating material applied on a unit area, or the area which may be covered by a unit volume. The layer of coating should be examined in cross-sections, usually by reflected light. A surface suitable for measurement may be obtained by grinding, or sometimes by fracture, in the case of hard materials (see page 154). Coatings on wood, leather; fabrics, or papers should be cut normal to the surface by means of a very sharp knife or razor blade (see page 149), so as to expose a smooth, undistorted cross-section for examination. Coatings on firm material may be cut free-hand, but softer objects or isolated coatings may need to be surrounded by a supporting material.[88] The cut surface is examined by reflected light, inclined illumination from a lamp with auxiliary condenser being most satisfactory, in the case of pigmented coatings. Examination of thin sections by transmitted light is not ordinarily necessary, except in the case of transparent coatings.

The number, color, evenness and relative thickness of successive layers of paint or other coating material may be readily observed, and careful measurements may be made at a number of points, to determine the average thickness and the magnitude of any variations. The area covered by one gallon of paint may be calculated, assuming a shrinkage of thickness of about one-third on drying; determinations of shrinkage due to evaporation may be made if the "spreading power" has already been determined experimentally.[89]

The weight per square foot of metal coatings such as electroplate may be calculated from thickness measurements, and quantitative studies of other types of coatings may be made in a similar manner.

[85] Abderhalden's *Handbuch der biochem. Arbeitsmethoden,* (Berlin, 1915), **8**, 495.

Metzner: *Das Mikroskop* (Leipzig, 1928), p. 403.

[86] *Berichte* **43**, 14 (1910).

[87] *Ind. Eng. Chem.* **17**, 974 (1925).

[88] Fonda: *Thesis.* p. 62, Cornell University (1925), used paraffin to support paint films prepared on tin foil. Maxwell: *Chem. Met. Eng.* **28**, 850, 964 (1923), removed the foil by amalgamation, and sectioned the film alone.

[89] Valuable data relative to the thickness of paint films may be found in **Circ. 71**, *Paint Mfrs. Ass. of U. S.* (Oct. 1919): Spreading Rates of Paint Products, by H. A. Gardner.

ANGULAR MEASUREMENTS[90]

The measurement of angles by means of the microscope is employed chiefly in the study of crystals, though it is also of value in other investigations. Angles in a plane normal to the axis of the microscope may be measured directly; their existence in this plane may be checked by noting whether the two sides are simultaneously and uniformly in focus. The measurement may be made most conveniently by means of a microscope equipped with a crosshaired eyepiece and a graduated rotating stage,[91] which should be carefully centered (page 273). The specimen is arranged so that the vertex of the angle to be measured is in the center of the field.

One side of the angle is aligned parallel and very near to one of the crosshairs, but not actually coincident with it. The graduations on the edge of the stage are read carefully, using a vernier if one is provided. The stage is now rotated until the other side of the angle is similarly aligned, and the reading repeated. The direction of rotation should preferably be such that the angle to be measured passes beneath the crosshair. The angle of rotation of the stage then gives the plane angle directly. Several readings on both sides of the angle should be made and averaged, if accurate results are essential.

If a rotating stage is not available, a goniometer eyepiece may be used. In such an eyepiece the crosshairs may be rotated, the angle being read by means of a graduated circular scale. Centering screws are provided, to render the axis of rotation coincident with the center of the field. The actual measurement is carried out just as with a rotating stage, except that the specimen remains stationary and the crosshair is rotated.

Angular measurements may also be made on tracings prepared by means of a drawing camera, or photomicrographs. An ordinary protractor or contact goniometer is used.

It should be borne in mind that angles measured between edges do not necessarily correspond to angles measured between *surfaces* which these edges intersect; in other words, that plane angles do not always equal interfacial angles. If the planes which meet at an angle are both parallel to the axis of the microscope, their angle of intersection can be measured directly. If they are inclined to the plane which they intersect, in which the measured angle lies, the true angle between them must be calculated by solid trigonometry (page 319).

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[90] See Johannsen: *Manual of Petrographic Methods* (1918), p. 293.

[91] The graduations of the stage are likely to be more accurate than the measurements obtained. They may be checked by a number of 180° settings of a straight-edged specimen with reference to one of the crosshairs.

Angles of slope, or angles between surfaces which meet in edges which are perpendicular to the axis of the microscope, may be measured by determining their coördinates in space by means of the eyepiece micrometer and the graduated fine adjustment. From the width of the projection of a given surface, measured laterally, and the vertical distance between its top and bottom edges, its inclination may be calculated trigonometrically.[92]

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[92] Dippel: *Handbuch der allgemeinen Mikroskopie* (Braunschweig, 1892), p. 907.

Hill: *Jour. Amer. Chem. Soc.* **48**, 651 (1926).

CHAPTER XIII

QUANTITATIVE ANALYSES OF HETEROGENEOUS MIXTURES

Some of the most difficult problems with which the chemist has to deal are those requiring a determination of the probable percentage composition or the amount of adulteration of materials which cannot be analyzed chemically, on account of their complexity or because the various ingredients would not be differentiated by ordinary analytical methods. As typical examples of these cases may be cited: mixtures of starches, meals, flours; adulterated spices, drugs, cocoa and other food products; mixtures in which " firsts " have been sophisticated with an inferior quality of the same material; paper pulps, fabrics, felts, ore concentrates, refractories, ceramic materials, concrete, rocks, alloys, and similar heterogeneous mixtures of materials of complex chemical composition.

A number of different microscopic quantitative analytical methods are available for the solution of problems of the above type. In order that these methods may be sufficiently accurate, the following requirements must be satisfied:

1 — The components of the mixture must differ sufficiently in microscopic appearance to permit easy recognition, or they must be capable of being rendered different by suitable methods of preparation or examination.

2 — The different components must bear a reasonably constant relationship to the physical or chemical properties in terms of which the analysis is ultimately to be interpreted.

3 — The specific gravities of the components must be nearly the same, or they must be known, or standards of known weight percentage composition must be available.

4 — The sample taken must represent the material to be analyzed, and the determinations must be subject to the usual criteria of dependable analyses; namely, that satisfactory checks be obtained from duplicate determinations, and from samples of known composition.

Distinction between the ingredients of a mixture may require only a glance, due to their naturally characteristic appearances. Unless these differences are very well marked, however, it is better to accentuate them by some additional treatment, in order that the components may be boldly evident, and that small quantities may not be overlooked. It is rare that any mixture is made up of materials which are uniformly typical and consistent in appearance, and it is well to guard against the possibility of mistaken identifications of portions with poorly defined properties.

Staining procedures are very useful in accentuating the contrast between the components of a mixture. Herzberg's[1] and other stains for paper and textile fibers, dyeing or chemical coloration of minerals (Fig. 153) and etching of alloys (Fig. 155), effect color changes of different character in the different ingredients, so that they are rendered unquestionably recognizable on inspection. Mounting in a medium of the proper refractive index often serves to differentiate between various substances which are otherwise similar. If the mounting medium is chosen so as to be of nearly the same refractive index as one of the components, and markedly different from the other, the first will be faintly outlined while the other will be heavily shaded (Fig. 154). The index of refraction of the mountant may well lie between those of the components, so that with oblique illumination they will be differently shaded, depending on whether they are of higher or lower refractive index. Minerals, crystals of inorganic and organic substances, certain textile fibers, may advantageously be mounted in this manner.

Methods of illumination are frequently very useful in emphasizing the differences between the various constituents of a mixture to be analyzed. Examinations between crossed nicols are particularly valuable, especially with crystalline materials or doubly refractive fibers or tissues. Fluorescence may serve to reveal heterogeneity which is not otherwise clearly apparent. Oblique or dark field illumination, or displacing the focus of the microscope slightly may serve to render one component more visible than another.

Sampling must be carried out with the usual precautions, and particular care should be taken to insure uniform mixing and to avoid any segregation of the different ingredients, either in collecting and reducing the sample or in mounting it. Powdered or

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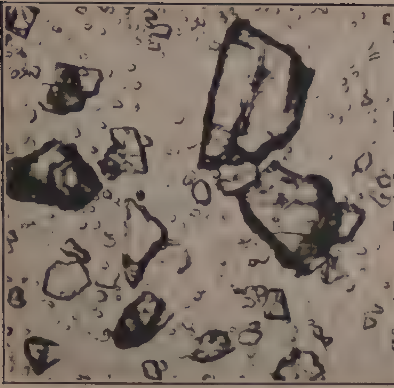
[1] The formula for this stain is given on p. 458.



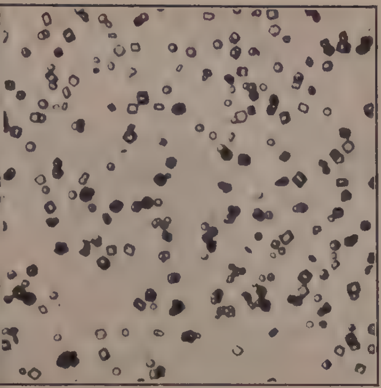


FIG. 150. Ground Limestone. 500×. FIG. 151. Precipitated Whiting. 500×.

Both these samples of calcium carbonate are "finer than 300 mesh."

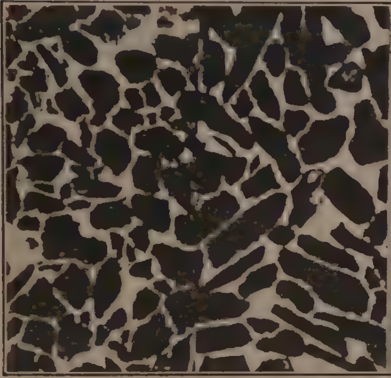


FIG. 152. Two Samples of Ground Stibnite, both of which passed through the same size sieve. Photographed by means of a comparison eyepiece.

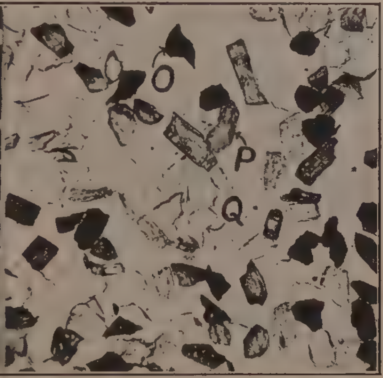


FIG. 153. Mixture of Ground Quartz *Q*, Orthoclase *O* and Plagioclase *P* Feldspars.

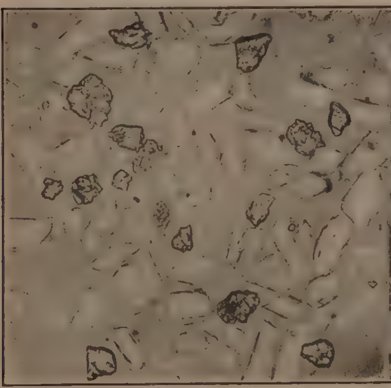


FIG. 154. Mixture of Ground Gypsum and Anhydrite. Mounted in a liquid of refractive index near that of Gypsum.

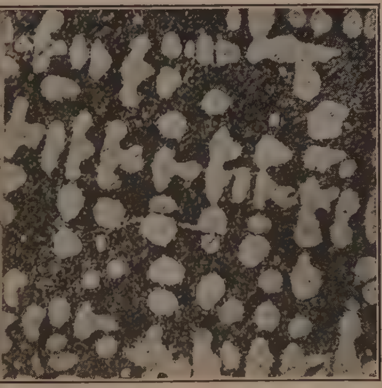


FIG. 155. Cast Copper, containing about 0.25 per cent Oxygen as *Cu-Cu₂O* Eutectic (dark).

fibrous materials must be handled with special caution and should preferably be suspended in a viscous liquid for uniform mixing, sampling and mounting. The concentration should be accurately known, since the final results must be calculated from it.

It is rarely possible to obtain perfectly consistent samples of materials which contain ingredients of widely different size, shape, or specific gravity, and for this reason results should not be based on a single preparation. Furthermore, since the distribution of the components in the specimen examined is not usually of ideal uniformity, examinations of several different fields should be made, and a large number of counts or measurements should be taken in each. By this procedure, averages of individual readings which appear hopelessly variant will show a reasonable and useful consistency.

The precautions and requirements just discussed imply the reasons why microscopic quantitative analyses are frequently less accurate than ordinary gravimetric or volumetric operations, on materials which can be sampled with a high degree of exactness. However, in most cases greater accuracy is not required, and could not be obtained by any method. Moreover, the accuracy of *interpretation* of microscopic analyses may far surpass that based on more precise determinations which at best can only be utilized in an approximate manner. For instance, it is useless to calculate the exact percentage of various minerals in an ore concentrate from its chemical analysis, if these minerals are variable in composition, or all contain the same elements. By virtue of the fact that the material itself is studied directly under the microscope, its true composition and many other significant features are revealed in an exceedingly positive and useable way. There is little chance for gross error in most microscopic analytical methods, for inspection will give an approximation of the correct result in most cases, and will serve as a check on faulty computations. The saving of time by the use of microscopical methods of analysis is frequently enormous, and may enable determinations to be made while the material is " in process."

It is on the above grounds that microscopic analysis of heterogeneous materials is recommended to the chemist, for they justify its trial in a great variety of problems, even where other methods are available. Its possibilities are far wider than is usually supposed, since they are not limited simply to determinations which cannot be made by any other means. The latter are of vital importance in certain types of work, particularly in the study of foods, papers, and textiles, but these constitute only a portion of the field of usefulness of microscopic methods.

METHODS BASED ON ESTIMATION

Method 1a: Estimation by Inspection. — If a specimen is properly prepared, so as to render its ingredients recognizable without any need of hesitation, it is frequently possible to estimate its composition with considerable accuracy. It is desirable that the components should be of approximately the same fineness and specific gravity, or the estimate may err in favor of the coarser or the bulkier material. However, most observers can judge to an accuracy of ± 10 per cent of the composition of simple mixtures containing two or three components. By continual practice on mixtures of the same components a much greater accuracy, comparable with that of results obtained by systematic counting or measurements, may be reached. In the analysis of papers, estimations by skilled microscopists are considered almost as exact as counts by the method given on page 442.[2]

Estimations may be employed in place of most of the methods to be described, with reasonable accuracy in the simpler cases. It is highly desirable that the analyst should continually test his judgment on mixtures of known composition, and thereby acquire a series of mental pictures which will serve as standards for comparison.

Method 1b: Comparison with a Series of Standards. — The accuracy of estimation methods can be increased if a series of mixtures of known compositions is available for comparison. It is usually fairly easy to determine between which of the standards the unknown lies, or to which it most closely approximates in composition. The comparison may be made by viewing the samples in succession, or better by means of a comparison microscope. For analyses based on estimations of areas, photomicrographs of standard samples may be used even by the novice.

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[2] Griffin: *Ind. Eng. Chem.* **11**, 968 (1919). See also:
Lee: *Paper Trade Jour.* **77**, No. 21, p. 51 (1923).
Tech. Asso. Pulp Paper Ind.: *The Manufacture of Pulp and Paper.* Vol. V (1925), Section 5, p. 52.

Reference to such pictures of specimens of known composition enables the result to be expressed in terms of the weight per cent of the desired component, rather than the areal per cent of the observed component. For instance, estimation of the carbon content of steel is possible to 0.1 per cent, although no free carbon is present, and the estimate is based on the amount of Pearlite present.[3] Similarly, the amount of oxygen in copper may be estimated to about 0.02 per cent, from the amount of $Cu - Cu_2O$ eutectic present[4] (Fig. 155).

Estimations by direct comparison with standards may be employed in almost all of the methods of microscopic quantitative analysis, especially if only two or three components are present, and if their distribution is uniform enough to give a representative appearance in every field.

METHODS BASED ON COUNTING

A wide variety of microscopical methods of quantitative analysis are based on some form of counting procedure. Numerical analyses are generally found more accurate than estimations, especially if several components are to be determined, if there is an unavoidable tendency to overestimate certain ingredients or if high magnification is necessary and the sample cannot be distributed uniformly enough for its composition to be truly represented by the small fields examined. The accuracy of the various methods can be considerably increased by counting a larger number of particles, but it must be borne in mind that no amount of averaging will compensate for poor initial sampling, or for errors in measuring the quantity of material to be counted.

Method 2a: Counting a Single Constituent. — The number of particles of a given component in a definite quantity of a mixture is frequently used as a measure of its quality. Particularly in the food industries such determinations are used for control and inspection, and yield information unobtainable by other methods.

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[3] Sauveur: *Metallography and Heat Treatment of Iron and Steel* (1926), Chap. V, p. 65.

[4] This procedure is employed as a rapid control method for the deoxidation of copper, samples being taken at intervals during the process, surfaced, etched, and examined within a period of about 10 minutes.

The determination of cocoa shells in cocoa is a typical example.[5] A 2-mg. sample is mounted in a clearing solution on a microscope slide. After sufficient transparency has been attained, the total number of shell fragments in the sample is counted. Comparison of the result with counts obtained on samples of known composition enables adulteration up to 10 per cent to be determined with an agreement to one or two per cent. Counts of bran particles or wheat hairs in flour are carried out in a similar manner, and give a measure of its grade.[6]

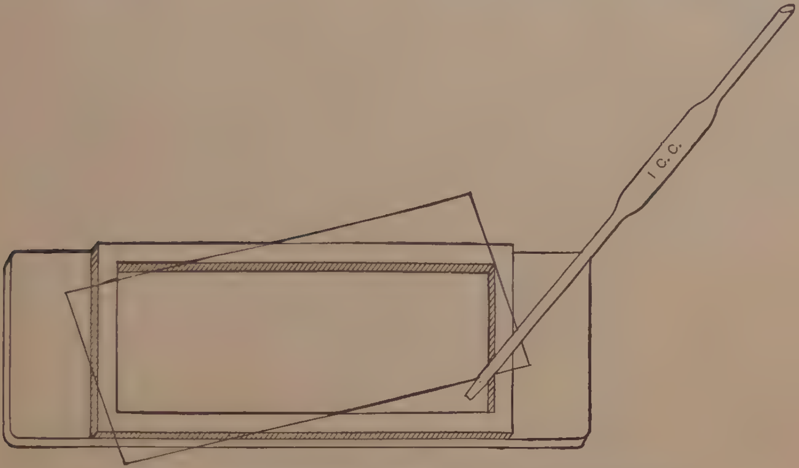

FIG. 156. Sedgwick-Rafter Counting Cell. (After Whipple.)

In order to insure systematic counting of the entire preparation, with no duplication or overlapping of fields, a mechanical stage (page 70) is essential.

Instead of counting all the particles of a component in a weighed sample of material, the quantity to be counted may be measured volumetrically. The Sedgwick-Rafter counting cell shown in Fig. 156 may be used. It consists of a brass rim cemented to a microscope slide, to give a cell 5 cm. × 2 cm. and 1 mm. deep, having a capacity of 1 cc. In the biological examination of water, the microscopic organisms are collected by filtration (page 148) and 1 cc. of a suspension (usually concentrated 100 times) is distributed evenly in the cell. The entire number of larger organisms is counted, and the numbers of smaller ones in several areas 1 mm. square are determined. By means of an eyepiece micrometer suitably ruled (Fig. 159) the inscribed field can be made to represent 1 mm.^{3} of liquid, by proper adjustment of the tube length.[7] Organisms of different sizes are "weighted" in the count according to their areas, as measured by the smaller squares of the micrometer.

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[5] Silberberg: *Jour. Ass. Off. Agr. Chem.* **6**, 98 (1922).
Pease: *idem.* **7**, 141 (1923); **8**, 176 (1924).

[6] Keenan and Lyons: *U. S. Dept. Agr. Bull.* **839**. (1922).
Keenan: *U. S. Dept. Agr. Bull.* **1130** (1923).

Smut spores in flour are determined by a similar method, by Bredemann: *Landw. Vers. Sta.* **75**, 134.

The determinations of a number of ingredients of stock feeds are described by Winter: *Mich. Agr. Coll. Expt. Sta. Spl. Bull.* **120** (1923).

Several useful quantitative methods for the examination of foods, drugs, and beverages are given by Schneider: *Microbiology and Microanalysis of Foods* (P. Blakiston's Son, Philadelphia, 1920) pp. 79–101; *Microanalysis of Powdered Vegetable Drugs* (1921) pp. 142–172.

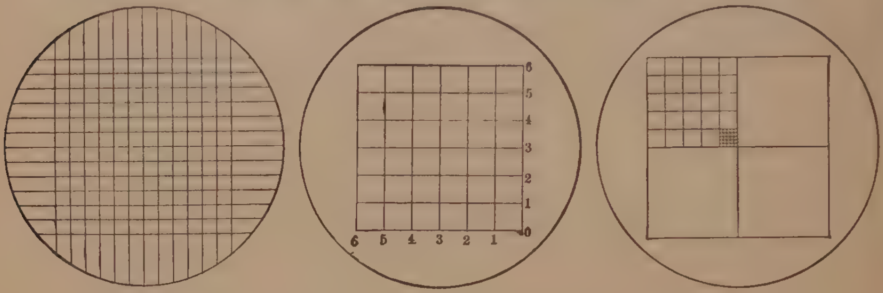

Fig. 157. Fig. 158. Fig. 159.

Net-Ruled Eyepiece Micrometers.

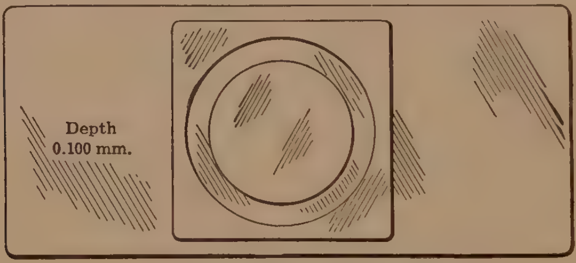

Fig. 160. Howard Counting Cell (Full size).

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[7] Whipple: *Microscopy of Drinking Water* (John Wiley & Sons, New York, 1927), pp. 95, 123.

Other types of cells may be used to measure the quantity of material which is counted. That of Howard[8] (Fig. 160) consists of a slide on which is cemented a plane circular glass plate about 20 mm. in diameter. Surrounding this raised portion, and separated from it by an annular space about 2 mm. wide, a glass frame is mounted. The thickness of the central plate is such that its upper surface is 0.100 mm. below that of the surrounding frame, so that a cover-glass resting upon the latter will leave a cell of this depth; special thick, plane cover-glasses are used. The cell serves to define the depth of the layer of liquid which is placed in it. The area in which a count is made may be bounded by the area of the field of the microscope or by a coördinate-ruled eyepiece micrometer; calibration is necessary in either case. Thus the volume of the liquid visible in the field may be accurately determined.

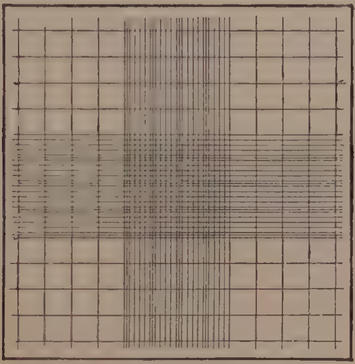

FIG. 161. Zappert-Neubauer Rulings of Haemacytometer Cell. 15×.

The area of the field may be regulated by a proper choice of lenses, or diaphragms of suitable opening may be cut out of black cardboard or thin metal, and dropped into the eyepiece.[9] The method of projecting a scale in the plane of the slide, by means of the condenser, is also useful for this purpose (see page 403). Systematic distribution of counts over all parts of the preparation is accomplished by means of a mechanical stage.

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[8] U. S. Dept. Agr. *Bull.* **581**, p. 21 (1917).

[9] The "Ehrlich" eyepiece of Leitz is provided with a variable, square diaphragm opening, for this purpose.

The Howard cell may be used for a wide variety of counting procedures on suspensions, emulsions, and powders which are brought into suspension. It is widely employed in the estimation of the mold content of tomato products; the original method[10] called for the examination of 50 fields, each 1.5 sq. mm. in area, and notation of the presence or absence of mold filaments in each. Revisions[11] recommend that the size of the mold filaments be taken into consideration in the count.

Various other types of counting chambers are available; of these, the haemacytometer cells used in making " blood counts " are most accurate and useful. They are essentially similar in construction to the Howard cell, except that the elevated areas are engraved; one type of ruling is shown in Fig. 161. The depth of the cell is usually 0.100 mm., and the area of the small squares is 0.0025 sq. mm. The newest haemacytometer cells are constructed of one piece of glass, without the use of cement. A thick, accurately plane cover-glass is necessary, to insure constant thickness of the film of liquid. The use of a ruled cell obviates any need for adhering to a constant magnification, and facilitates systematic counting of fields from all parts of the preparation. Care should be taken in cleaning the cell and gritty specimens should not be placed in it, in order to avoid scratching the delicate rulings.

Another method of measuring the quantity of material which is counted is to prepare a uniform suspension, spread a given amount over a definite area, and make counts on an aliquot portion of this area.

This procedure is followed by Breed and Brew[12] in their rapid method for the direct bacterial count of milk, cheese, soils, and other materials. By means of a capillary pipette, 0.01 cc. of milk or other suspension is collected and deposited on a clean microscope slide, being spread evenly over an area of 1 sq. cm. After drying, extracting fat, and staining, the film is examined by means of a high power objective, and the bacteria are counted. If the magnification is properly adjusted, a simple relationship between the area of the field and the volume of the preparation may be obtained. For instance, if the diameter of the field is 0.16 mm. its area represents approximately one-five thousandth of the sample, and each bacterium seen in a field is equivalent to 500,000 per cc. of milk.

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[10] Howard: *loc. cit.*

[11] *Ass. Off. Agr. Chem. Methods.* **1920**, p. 164.

Jour. A.O.A.C. **6**, 146 (1922).

[12] *N. Y. State Agr. Expt. Sta. Techn. Bull.* **49** (1916); *Circ.* **58** gives an extensive bibliography of applications and allied methods.

Essentially the same principle may be employed in the analysis of powdered mixtures, without the need for the numerical determination of the size of field or the quantity of sample taken, if a curve is plotted from counts on samples of known composition, prepared under identical conditions.[13]

Standard mixtures, the percentage composition of which is definitely known, must first be prepared, and rendered thoroughly uniform by mixing. At least three such mixtures should be available, so that the entire range of possible compositions will be covered.

From each of these standards, samples of equal size are taken. The ideal method of sampling is to suspend the material in a viscous liquid, mixing thoroughly and pipetting out a small quantity before any settling occurs. Mixtures of glycerine, glucose, gums, dextrin, gelatine, or mucilage in water, are suitable for suspending most materials; heavy oils or Canada balsam may also be used. Care should be taken to avoid bubbles, lumps, or imperfect wetting of the powder, when the suspensions are made up.

Weighing may be used for dry material; almost unweighably small quantities of fine powders may be prepared by spreading out a weighed amount of dry material on a piece of glazed paper or glass, to give a thin uniform layer of square or circular shape. This is " quartered " and the operation repeated if necessary, until a portion equivalent to a few milligrams is obtained.[14] An even better method depends on " diluting " the powdered material with several times its weight of a finely divided soluble material such as sucrose, lactose, dextrose, or soluble dextrin. After thorough mixing, a small quantity is weighed out and transferred to a microscope slide. The mounting liquid is added, and the diluent material dissolves, leaving a known weight of the insoluble sample.[15]

Fairly uniform samples of dry materials which are easily compacted, such as starch and other fine powders, may be measured by volume. A small brass rod, with a shallow hemispherical depression (1.5 mm. in diameter, 0.5 mm. deep) in one end may be advantageously employed.[16] The material to be measured out is spread in a thin layer on a piece of glazed paper or glass and the sampling rod is pressed gently upon it. By a sliding motion the excess powder may be removed from beneath the end of the rod, and on lifting it up a tiny pellet will be retained in the depression, which should be " level full." Any surplus powder may be wiped off by the finger tip, and the pellet may then be dislodged on to a carefully cleaned slide by a light tap.

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[13] Chamot: *Seventh Int. Cong. Appl. Chem.* Section VIII C, 249 (1909).

[14] Kraemer: *Jour. Amer. Chem. Soc.* **21**, 659 (1899).

[15] Hartwich and Wichmann: *Arch. Pharm.* **250**, 450–71.

It is mixed thoroughly with a small quantity of a mounting liquid, such as glycerin and water (1 : 1); just sufficient liquid should be used to spread over the area covered by the cover-glass used. The mixing may be accomplished by stirring with a fine glass rod, or the cover-glass may be touched to the drop of liquid and moved gently up and down, without being allowed to settle and spread out the liquid. The cover-glass must be scrupulously clean. After mixing is complete, as shown by a lack of any streaks in the suspension as it is held over a dark background, the suspension is spread out to give a uniform film, by allowing the cover-glass to settle down gently upon it. If bubbles have been avoided in the mixing, and if the slide and cover-glass are free from grease, the liquid will flow evenly, and the suspended material will be well distributed over the area covered by the cover-glass, with no surplus liquid pressed out at the edges. If the dispersion is uneven, as shown by examination with a low power, it is inadvisable to make counts upon the preparation, and another should be prepared. At least three slides should be made of each mixture examined, in order to compensate for unavoidable inaccuracy in measuring out the dry material.

If the same sampling rod and the same sized cover-glasses are used throughout, the portion of each sample included in the area of the field of the microscope will be the same. It is not necessary to know the absolute amount of material within the field, as long as this amount is kept constant. Obviously, the objective, eyepiece and tube length must remain unchanged during the series of counts.

The number of particles of the adulterant or minor constituent is counted in 15 or 20 fields on each slide. A mechanical stage is exceedingly useful for insuring that these fields are well distributed over the preparation, and a net-ruled eyepiece micrometer (Fig. 158) is desirable to facilitate counting.

The operation is repeated for each of the standard mixtures, and the average number of particles counted in a field is plotted against the known per cent of that component, as shown in Fig. 162. The curve should be a straight line passing through the origin. An unknown mixture to be analyzed is prepared and counted in precisely the same manner, and its composition is then read off from the curve.

Although the above method appears to be liable to many errors, it has been found very satisfactory even in the hands of beginners. The results are less accurate when large amounts of adulterant are present, but usually agree to within ± 10% of the value determined.

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[16] Private communication from Dr. H. S. Booth of Western Reserve University.

Method 2b: Utilization of Ratios. — Analyses of mixtures containing more than two components require that each should be counted, and this is often advisable in simpler mixtures. The preliminary preparation of a curve from known mixtures, and the necessity of measuring out the same amount of material for each count, are eliminated. This usually saves more time than that required for counting the additional constituents.

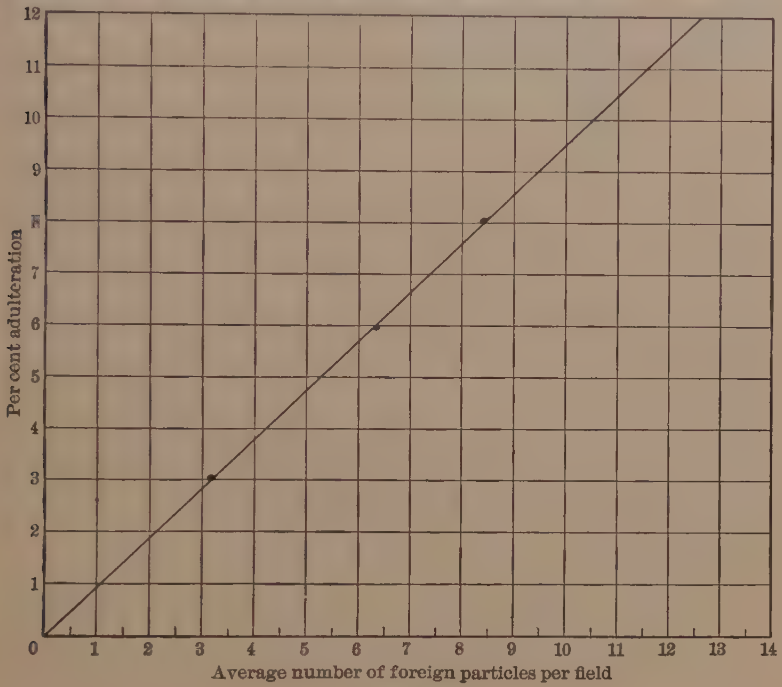

FIG. 162.

The ratio to one another of the amounts of different constituents present in various powdered foods and drugs is frequently a measure of the quality and purity of the material. In case a certain essential and typical kind of tissue is abnormally low in quantity, the sample is to be viewed with suspicion, even if foreign material is not recognized. The method of examination recommended by Schneider[17] is to spread out the material on a slide or in a counting cell, and to count all the different characteristic fragments of tissue in at least fifty fields. The average number of each is tabulated, and compared with the relative numbers of similar tissues in a specimen known to be of satisfactory grade.[18]

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[17] *Microanalysis of Powdered Vegetable Drugs* (1921), pp. 154–173.
See also Meyer: *Zeits. Untersuch. Nahr. Genussmitt.* **17**, 497 (1909).
Ezendam: *idem.* **18**, 462 (1909).
Herter: *idem.* **38**, 65 (1919).

An ingenious method involving ratios has been devised by Wallis.[19] A **reference substance** containing a known number of particles per gram, is added in definite amount to a given weight of powdered sample, and thoroughly mixed with it in a suspension. A portion of the mixture (not measured) is spread out on a slide, and particles of both materials are counted. The number of particles of the reference substance is a measure of the actual weight of sample being counted, and this method is useful as a substitute for weighing or measuring out a small sample for study. On account of its uniformity, lycopodium powder is recommended as a reference substance; it contains 94,000 particles per milligram.

To give a specific example: Equal weights of lycopodium powder and a mixture of known percentage composition (10 per cent potato starch and 90 per cent rice starch, as in Method 2a, page 438) are mixed, and the particles of lycopodium and of rice starch are counted in ten fields from each of three slides. A similar 1 : 1 mixture, containing rice starch and potato starch in unknown relative percentages is now counted. Suppose that the unknown mixture contains twice as many particles of potato starch *per particle of reference substance* as did the 10 per cent mixture; its composition is evidently 20 per cent potato starch, 80 per cent rice starch.[20]

Method 2c: Counting All Constituents. — If all the grains of each component are counted, measurements of the amount of sample by volume, weight, or a reference substance, are unnecessary. Analyses of this type are very commonly employed, especially in the examination of papers, textiles, and powdered mineral materials.

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[18] A number of applications of numerical information of this character are given by Schneider: *loc. cit.*, and by Wallis: *Analytical Microscopy* (E. Arnold, London, 1923), p. 135.

[19] Wallis: *Analyst* **41**, 357 (1916); *Pharm. Jour.* **103**, 75 (1919); *op. cit.*, Chap. XI.

Liversage: *Analyst* **47**, 430 (1922).

[20] This method has been applied to the direct bacterial count of milk, by using an admixture of blood as a reference substance, since the number of corpuscles in a given volume of normal blood is practically constant. Pozzi-Escot: *Ann. chim. anal.* **5**, 130 (1923).

The determination of the content of quartz in ground feldspars used for ceramics is of considerable importance, and is not accurate or convenient by purely chemical means. A number of microscopic methods have been worked out, all of which depend first of all on some means of rendering the quartz particles easily distinguishable from the feldspar. Rapid fusion of the feldspar to a glass, without affecting the quartz, immersion in a liquid of the same index of refraction as the glass, and comparison of the amount of quartz grains with standard samples, is recommended by Booze and Klein.[21] Insley depends on immersion of the untreated powdered minerals in a liquid of refractive index 1.540, which is below that of quartz and above that of feldspar. About 200 grains, on each of three slides, are counted.[22]

A better method, which also differentiates between orthoclase and plagioclase feldspars, utilizes a preliminary attack by hydrofluoric acid vapor. This etches the feldspars, but leaves the quartz clear; treatment with $Na_3Co(NO_2)_6$ solution colors the potassium feldspar grains yellow[23] (Fig. 153).

Mixtures of gypsum and anhydrite, with minor quantities of other minerals, are analyzed in a similar manner (Fig. 154). Larsen[24] uses a mounting medium of refractive index between those of gypsum and anhydrite, and counts the grains of both minerals, taking into consideration the size as well as the number of the particles. If the material is previously graded by means of sieves, to give a powder of uniform particle size, counting alone is sufficient to give the *volume* percentage composition of the sample. Determinations of the composition *by weight* require that the specific gravities of the constituents be taken into account.

Ore concentrates are frequently complex in composition and difficult to analyze by chemical means, particularly since the actual amounts of the different minerals, rather than of the elements, are of interest. The effectiveness of the grinding may be judged by the proportion of grains consisting of more than one mineral, as determined by counting.[25] The degree of separation of the different kinds of mineral grains at different stages of the concentrating process may be followed by microscopic determinations based on the number and size of the various particles.[26] Such examinations may be carried out at fairly low magnifications, by means of a Greenough binocular microscope or the Ore Dressing Microscope of Leitz. The material is spread over a surface which is marked off into squares to facilitate systematic counting.

[21] *Jour. Amer. Ceram. Soc.* **6**, 698 (1923).

[22] *Jour. Amer. Ceram. Soc.* **10**, 651 (1927).

[23] Gabriel and Cox: *Amer. Mineral,* **14**, 290 (1929).

[24] *Proc. A.S.T.M.* **23, I**, 236 (1923).

Gardner: *idem.* **26, I**, 296 (1926).

[25] Hynes: *Min. Sci. Press.* **110**, 994 (1915).

[26] Campbell: *Eng. Min. Jour.* **103**, 929 (1917).

Thomas and Apgar: *Chem. Met. Eng.* **18**, 514 (1918).

Coghill and Bonardi: *Bur. Mines. Tech. Paper* **211** (1919).

Schneiderhöhn: *Anleitung zur mikroskopischen Bestimmung und Untersuchung von Erzen und Aufbereitungsprodukten* (Berlin, 1922), pp. 32–39.

Herzog[27] has suggested a numerical method for the analysis or textiles composed of a mixture of different fibers. The sample (fabric, yarn, cord, etc.) is cut into small pieces so as to give bits of fiber about 1 mm. long. This cutting may be done free-hand with scissors, or the sample may be embedded in paraffin and 1 mm. sections cut with a microtome. The mixture of short pieces of fibers is spread evenly over a slide, either by suspending in warm gelatine solution or by melting the paraffin. The entire number of each kind of fiber is then ascertained, and from their known sizes and specific gravities the weight per cent of each may be computed.

Quantitative analyses of papers are very commonly made on the basis of microscopic counts. The procedure recommended by the Technical Association of the Pulp and Paper Industry is essentially as follows:[28] The sample is pulped by boiling in 0.5–1 per cent sodium hydroxide solution, washed several times on a 200-mesh sieve, and the suspension is diluted to about 0.1 per cent. A few drops are transferred to a slide by a 6-mm. glass tube, and spread evenly. After drying by " blotting," or in an air bath, the stain (usually Herzberg's, page 458) is applied and the preparation is covered with a cover glass. Using a magnification of 100×, the amount of each kind of fiber is determined in 10–25 fields. Since the fibers vary widely in length, their size must be taken into account by estimating their length in terms of the diameter of the field, and counting the *total number of diameters* corresponding to each kind of fiber, rather than the number of fibers. Allowance is also made for the differences in the weights of fibers of different varieties, the numerical quantities being multiplied by weight factors[29] to permit the weight percentage composition to be computed.

A novel method of counting, which is applicable to various sorts of numerical analyses, is described by Lofton.[30] It is particularly suitable for fibrous materials such as papers and roofing felts,[31] since it eliminates the need for taking the length of the fibers into account. This " dot-count " method consists in moving the preparation slowly beneath the microscope, by means of a mechanical stage. As the fibers travel across the field, each one, of which any part passes beneath the intersection of the crosshairs of the eyepiece, is counted; the longer fibers may thus be counted more than once. Counts are taken in different places and directions through the preparation, so that at least 200 fibers on each of three slides are counted.

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[27] *Koll. Zeits.* **1**, 202 (1907); *Textile Forsch.* **2** (1922); *Zeits. wiss. Mikros.* **39**, 357 (1923); *Mikroskopische Untersuchung der Seide und Kunstseide* (Berlin, 1924), pp. 16–19.

[28] T.A.P.P.I.: *Manufacture of Pulp and Paper*, Vol. V (1925), Sec. 5, p. 49. See also

Sutermeister: *Chemistry of Pulp and Paper Making* (1920), p. 386.
Herzberg: *Papierprüfung* (Berlin, 1927), p. 124.
Spence and Kraus: *Paper* **20**, 11 (1917).
Lee: *Paper Ind.* **4**, 928; *Paper Tr. Jour.* **75**, 47 (1922).
Dickson: *Analyst* **48**, 372 (1923).
Reed and Machmer: *Paper Tr. Jour.* **76**, 47 (1923).
Wightman: *Eastman Kodak Co. Sci. Pub.* **7**, 9 (1924).
Bright: *Pulp Paper Mag. Can.* **24**, 615 (1926).

[29] Spence and Kraus: *loc. cit.*
Sutermeister: *op. cit.*, p. 392.

[30] *Paper Tr. Jour.* **84**, 57 (1927).

[31] A.S.T.M.: *Tentative Method of Analysis of Roofing Felt for Fiber Composition*, **D 272–27 T.**

As an alternative procedure, every fiber or portion of a fiber which is intercepted by one of the crosshairs may be counted without moving the slide. This is repeated in a number of different fields.[32]

METHODS BASED ON AREAL OR LINEAR MEASUREMENTS

All the methods of this class depend on the fact that if a plane or a line is passed through an aggregate of heterogeneous material orientated at random, the total areal or linear intercepts of each constituent with that plane or line are proportional to the volumes of the respective constituents. A thorough critical study of the accuracy of such methods has been made by Alling and Valentine.[33]

Areal or linear analyses are most appropriately carried out on compact materials which may be surfaced or sectioned easily; for instance, alloys, rocks, refractories, concrete, etc. Fragmental materials, such as ore concentrates, may be bonded together for surfacing by embedding in sealing wax or other cement.[34] The fundamental principle of the methods does not apply accurately to material in the form of loose grains which are not sectioned, especially if the particles are of a wide variety of sizes.[35] Soft porous materials such as mixtures of various kinds of plant tissues, are better analyzed by the methods previously described.

Method 3a: Areal Analysis. — The areas of the various constituents intersected by the plane of the surface or thin section used are measured by any of the methods given on page 421. If a planimeter is not available, the areas may be traced on heavy paper by means of a drawing camera, or photographed, and the various constituents cut out and weighed. The simplest method is to trace the outlines of the components on a piece of coordinate paper (preferably ruled in 5-mm. squares) and total the areas of each by counting the squares covered. If a mixture of two constituents is examined, only one of these need be measured, the other being determined by difference. If a powdered mixture is measured, the embedding material is of course ignored. The magnification used will depend upon the fineness of grain of the specimen. In general, the lowest power that will permit accurate outlines should be employed; and the field should be large enough to include at least ten grains of the minor constituent. At least ten fields should be measured; more may be necessary if the specimen is not of ideally uniform composition throughout. In critical work enough determinations should be made to yield averages which check satisfactorily.

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[32] Geohegan: *Paper Tr. Jour.* June 6, **1929**, p. 63.

[33] *Amer. Jour. Sci.* **14**, 50–65 (1927).

See also Johannsen: *Manual of Petrographic Methods* (1918), pp. 290–2.

[34] Head: *Jour. Franklin Inst.* **192**, 250 (1921).

Thomson: *Amer. Mineralogist* **8**, 99 (1923).

[35] Satisfactory results have been obtained on rock dusts without sectioning, by Emery and de Chicchis: *Carnegie Inst. Tech. Min. Met. Inv. Bull.* **21** (1925).

The results obtained from the above areal measurements give percentage composition *by volume.* Only in case the components are of practically identical specific gravities does this analysis give percentage composition by weight. Two methods are available for determining the latter.

A — The volume per cent of each component may be multiplied by its specific gravity and the resulting ratios computed in terms of weight per cent. The results of such a calculation represent the relative amounts of the different solid components which are present, but do not necessarily give the chemical analysis of the sample. If the components are of constant composition, the chemical analysis may be calculated if desired, but it is not ordinarily of direct importance in the examinations for which this method is chiefly employed.

B — The volume percentages of each component may be multiplied by the respective percentages of a given element contained in them, and totalled to give the weight per cent of that element in the mixture. This method of calculating results is commonly used in determining the composition of alloys. For example, if a sample of copper exhibits an areal composition of about 60 per cent Cu–Cu_2O eutectic (Fig. 155) and if the eutectic contains 0.39 per cent oxygen, the oxygen content of the sample is 0.25 per cent.[36]

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[36] The use of areal analyses in this determination is discussed by Bardwell: *Bull. Amer. Inst. Min. Eng.* **79**, 1429; *Trans. Amer. Inst. Min. Met. Eng.* **46**, 742 (1913).

Areal analyses for the determination of the relative volumes or weights of constituents of mixtures are particularly valuable in cases where chemical analyses would not indicate the quantities present. The following additional examples are of interest because of the methods used as well as their applications: concrete,[37] alloys,[38] ores,[39] rocks.[40]

Method 3b: Linear Analysis. — Instead of measuring areal intercepts on the plane through the specimen, the linear intercepts on a line across its surface are measured.[41] Various procedures may be employed for measuring and totalling the linear intercepts corresponding to the grains of each separate constituent. An eyepiece micrometer may serve for fine grained materials, but is not very convenient. Lines may be drawn in different places and directions across drawings or photomicrographs of the specimen, and the intercepts on them measured by means of a scale. A simple method of totalling is to use a separate strip of paper for each constituent, marking off on it the successive intercepts of that material; the total lengths are easily measured by a scale, and compared in terms of volume percentages.[42]

Mechanical devices also facilitate the measurements greatly. A filar micrometer is useful for fine grained material, and a graduated mechanical stage may serve for coarse grained specimens. The two may be used jointly, in the analyses of mixtures of two components; the minor one is measured by means of the filar micrometer, and the other by means of the mechanical stage. Each intercept is not measured numerically, but is moved beneath the crosshair and the respective totals read from the scales of the micrometer and mechanical stage. The special recording micrometers referred to on page 397 are much more convenient and permit totalling the measurements for several constituents. The successive grains of a component are moved across beneath the crosshair by a micrometer screw, different screws being used for each component, so that the final readings on their respective scales correspond to the totals of the various intercepts.

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[37] N. C. Johnson: *Eng. Rec.* Feb., 27 (1915).

[38] Mathewson: *Chem. Met. Eng.* **11**, 619 (1913).
Jeffries: *idem.* **11**, 668 (1913).
Logan: *Phot. Jour.* **49**, 9 (1925).

[39] Wagemann: *Metall u. Erz* **24**, 52 (1927).

[40] Johannsen: *loc. cit.*

[41] This method is sometimes named after its originator, Rosiwal: *Verh. geol. Reichanstalt* **1898**, pp. 143–75.

[42] Richardson: *Min. Mag.* **19**, 314 (1922).

The number of fields which must be measured for accurate results is considerably larger than in the case of areal analysis. According to Johannsen and Stephanson,[43] the total distance measured should be at least 100–200 times the diameter of the average grains, and the lines on which intercepts are measured should not be closer together than the width of one grain. If there is any lack of uniform distribution in the sample, more measurements, in a greater variety of positions and directions, should be taken. It is preferable to measure all constituents in one operation, so that they may be used as checks against each other.

The discussion of the calculation of results of areal analyses applies equally to linear analyses, and the applications of these two types of methods are, in general, the same. The procedures employed in the studies of refractories,[44] and alloys[45] throw additional light on the technique of linear analysis.

[43] *Jour. Geol.* **27**, 212 (1919).

[44] Insley and Klein: *Bur. Stds. Tech. Paper* **124**.

Scott: *Tr. Eng. Ceram. Soc.* **17**, I, 188 (1917).

[45] Poluskin: *Trans. Amer. Inst. Min. Met. Eng.* **71**, 669 (1925).

APPENDIX

REFERENCE BOOKS ON APPLIED MICROSCOPY

In addition to the works cited in the chapters devoted to discussions of general principles and methods the following are given as particularly useful references on the applications of microscopy to special fields.

Microscopic Qualitative Analysis, and Microchemical Manipulations

Behrens-Kley, *Mikrochemische Analyse* (Voss, Leipzig, 1915).

Behrens-Kley, *Organische Mikrochemische Analyse* (Voss, Leipzig, 1922).

Donan, *Arbeitsmethoden der Mikrochemie* (Franckh'sche Verlagshandlung, Stuttgart, 1913).

Emich, *Methoden der Mikrochemie*, in Abderhalden's *Handbuch der biologischen Arbeitsmethoden*, Abt. I, Tl. 3, Hft. 1, pp. 45–324 (Urban & Schwarzenberg, Berlin, 1921).

Emich, *Mikrochemisches Praktikum* (J. F. Bergmann, Munich, 1924).

Emich, *Umsetzungen sehr kleiner Stoffmengen* (*Mikrochemie*), in Stähler's *Handbuch der Arbeitsmethoden in der anorganischen Chemie*, Bd. II, Tl. 2, pp. 655–882 (W. de Gruyter, Berlin, 1925).

Emich, *Lehrbuch der Mikrochemie* (J. F. Bergmann, Munich, 1926).

Klein and Strebinger, editors, *Fortschritte der Mikrochemie* (Fr. Deuticke, Leipzig, 1928). An extensive symposium covering the years 1915–1926.

Mayrhofer, *Mikrochemie der Arzneimittel und Gifte*, Parts I and II (Urban & Schwarzenberg, Berlin, 1928).

Textile and Paper Fibers

Beauverie, *Les Textiles Végètaux* (Gauthier-Villars, Paris, 1913).

Herzberg, *Papierprüfung* (J. Springer, Berlin, 1927).

Herzog, *Mikrophotographischer Atlas der technisch wichtigen Textilfasern*, I. Teil (Obernetter, Munich, 1908).

Herzog, *Mikroskopische Untersuchung der Seide und Kunstseide* (J. Springer, Berlin, 1924).

Herzog, *Die Unterscheidung der Flachs- und Hanffaser* (J. Springer, Berlin, 1926).

von Höhnel, *Mikroskopie der technisch verwendeten Faserstoffe* (A. Hartleben, Leipzig).

Laurie, *Textile Microscopy* (E. Benn, London, 1928).

Mathews, *Textile Fibers* (John Wiley & Sons, New York, 1924).

Sutermeister, *Chemistry of Pulp and Paper Making* (John Wiley & Sons, New York, 1928).

Technical Association of Pulp and Paper Industry, *The Manufacture of Pulp and Paper*, Vol. V (McGraw-Hill Book Co., New York, 1929).

Foods, Drugs, and Sanitary Chemistry

Barnstein, *Anleitung zur mikroskopischen Prüfung und zur Begutachtung der Kraftfuttermittel* (Borntraeger, Berlin, 1920).
Clayton, *Compendium of Food Microscopy* (Wm. Wood & Co., New York, 1909).
Greenish, *Microscopical Examination of Foods and Drugs* (P. Blakiston's Son, Philadelphia, 1910).
Greger, *Mikroskopie der landwirtschaftlichen Unkrautsamen* (P. Parey, Berlin, 1927).
Kinzel, *Mikroskopische Futtermittelkontrolle* (E. Ulmer, Stuttgart, 1918).
Koch, *Die mikroskopische Analyse der Drogenpulver* (Borntraeger, Leipzig, 1901).
Koch, *Einführung in die mikroskopische Analyse der Drogenpulver* (Borntraeger, Berlin, 1906).
Macé, *Les Substances Alimentaires étudiées au Microscope* (Baillière, Paris, 1891).
Moeller-Griebel, *Mikroskopie die Nahrungs- und Genussmittel aus den Pflanzenreiche* (J. Springer, Berlin, 1929).
Morris, *Microscopic Analysis of Cattle-Foods* (Cambridge Univ. Press, 1917).
Schneider, *Microbiology and Microanalysis of Foods* (P. Blakiston's Son, Philadelphia, 1920).
Schneider, *Microanalysis of Powdered Vegetable Drugs* (P. Blakiston's Son, Philadelphia, 1920).
Scott, *The Microscope in the Mill* (Northern Pub. Co., Liverpool, 1920)
Ward and Whipple, *Fresh Water Biology* (John Wiley & Sons, New York, 1918).
Whipple, *Microscopy of Drinking Water* (John Wiley & Sons, New York, 1927).
Winton, *Microscopy of Vegetable Foods* (John Wiley & Sons, New York, 1916).

Miscellaneous Technical Applications

Erdmann-König, *Warenkunde*, 2 Vols. (J. A. Barth, Leipzig, 1925).
Hager-Tobler, *Das Mikroskop und seine Anwendung* (J. Springer, Berlin, 1925).
Hanausek-Winton, *Microscopy of Technical Products* (John Wiley & Sons, New York, 1916).
Molisch, *Mikrochemie der Pflanze* (G. Fischer, Jena. 1921).
Poschl, *Technische Mikroskopie* (Stuttgart, 1927).
Sasserath, *Mikroskopische Warenprüfung* (S. Hirzel, Leipzig, 1910).
Schneider-Zimmermann, *Botanische Mikrotechnik* (G. Fischer, Jena, 1922).
Tunmann, *Pflanzemikrochemie* (Borntraeger, Berlin, 1913).
Wallis, *Analytical Microscopy* (Edw. Arnold & Co., London, 1923).
Wilson, *Chemistry of Leather Manufacture* (Chemical Catalog Co., New York, 1923, 1928).

Journals

Journal of the Royal Microscopical Society, 20, Hanover Square, London, W. 1.
Zeitschrift für wissenschaftliche Mikroskopie, Published by S. Hirzel, Leipzig.
Mikrochemie, Published by E. Haim & Co., Wein and Leipzig.

SYNOPSIS OF LABORATORY PRACTICES IN INTRODUCTORY CHEMICAL MICROSCOPY — CORNELL UNIVERSITY DEPARTMENT OF CHEMISTRY

I. MICROMETRY — CHAPTER XII

1. *Calibration of Eyepiece Micrometer Scale* (see page 398). — Prepare in your notebook a table similar to the following:

Microscope No....... .....× Micrometer Eyepiece

Objective	Tube Length	Eyepiece Micrometer Divisions = Stage Micrometer Divisions	Eyepiece Micrometer Divisions = 0.1 mm.	1 Eyepiece Micrometer Division =
32 mm.		=		μ
16		=		μ
8		=		μ

2. *Estimation of Weight* (see page 422). — Measure at least three different diameters of a metal bead, with each of the objectives on your microscope. Record all readings, and calculate the diameters in microns, as measured by each different objective. The values obtained will serve as a check on the calibration of the eyepiece micrometer.

Average the results and compute the weight of the bead, in grams.

3. *Thickness of Protective Coatings* (see page 424). — Prepare a specimen of paint films or other protective coatings, so as to present a smooth, cross-section perpendicular to the surface (page 149). Tabulate the following data for each coat, in the order of their application: color, relative thickness, uniformity of thickness. Measure the third coat carefully in a number of places, and calculate its thickness in millimeters and in inches. Calculate the "spreading power" of this paint, assuming no shrinkage, in square feet per gallon.

II. EXAMINATION OF POWDERED MATERIALS (see page 408)

1. Examine a series of preparations of fine abrasive powders, by means of the comparison microscope (page 68), arranging them in order with respect to: (*a*) fineness, and (*b*) uniformity. Note the general shape of the particles in each sample, and draw what conclusions you can as to the mechanical and abrasive properties of the materials.

2. Mount samples of several common pigments and fillers, in glycerine and water (1 : 1), as directed on page 169. Examine the materials and compare them with each other, noting their fineness, uniformity, tendency to aggregate, and any other distinctive features. How do the microscopic characteristics of powders prepared by grinding differ from those of precipitated materials?

3. Examine a piece of the finest standard testing sieve, 325 meshes per inch (see page 410). What is the diameter of the openings, in microns? How

much do they vary? How does the size of the openings compare with the order of magnitude of the above pigments and fillers?

4. *Importance of Particle-Shape in Sieve Tests.* Consult the specifications posted on the bulletin board.

a. Spread some powdered antimony sulphide (ground stibnite) uniformly upon a microscope slide. Using the drawing camera, prepare drawings of several different fields of particles, to scale (page 395). Draw a portion of a "100-mesh" sieve (opening 147 μ) to the same scale.

b. Explain, by means of drawings, why the actual volume or weight of particles such as these is not directly measured by the size of the sieve openings which pass or retain them.

c. Given a material which breaks up into elongated particles on grinding, how would you determine the proper sieve opening to specify, in order to obtain particles having a given maximum volume?

III. GREENOUGH BINOCULAR MICROSCOPE (see page 62)

Examine the mechanical and optical features of the instruments, noting particularly the stereoscopic and erect image, long working distance, great depth of focus, and flexible stands.

Practice sorting out the different ingredients of a mixture of powdered materials.

Try removing samples of inclusions or surface coatings, without contaminating them with adjacent material.

IV. QUANTITATIVE ANALYSES OF HETEROGENEOUS MIXTURES — CHAPTER XIII

1. *Estimation* (see page 431).

a. Examine a stained preparation of a paper pulp containing a mixture of different kinds of fibers. Estimate the percentage of each in the sample.

b. Compare with samples of similar mixtures having known compositions.

2. *Counting* (see page 432).

a. Counts of one constituent (see page 432). Prepare 3 slides of a mixture of rice starch containing 5 per cent potato starch, as directed on page 437, and count the number of potato starch grains in at least 15 fields on each slide. Use a mechanical stage to insure systematic distribution of the fields counted. Make similar counts on 10 per cent and 20 per cent mixtures, and plot the results as a curve.

Obtain an unknown mixture, and determine its composition.

b. Counts of all constituents (see page 440).

Mixture of quartz and feldspars (page 441). — The powdered mixture (100 to 140-mesh) is mounted in a medium of $n = 1.48$. Using a 32 mm. objective and net-ruled eyepiece, tabulate the number of particles of each mineral in the preparation, using a mechanical stage to insure systematic counting. Determine the *numerical* per cent of each ingredient in the mixture. Assuming the same average fineness for all three minerals, what would be the volume percentage composition? Since their specific gravities are nearly identical, the composition by volume will be equivalent to that by weight.

Mixture of gypsum and anhydrite (page 441). — Distribute the powdered mixture (100–120 mesh) uniformly in a liquid having a refractive index of 1.550, and cover with a cover-glass. Learn to differentiate the two kinds of particles (gypsum — faint brownish outline, platy cleavage; anhydrite — bold black outline, imperfect cleavage). Use a 32-mm. objective and a net-ruled eyepiece, and tabulate the number of particles of each mineral, in 20 fields on each of at least two preparations. Determine the *numerical* percentage composition. Assuming uniform fineness, what would be the *volume* per cent of each mineral? Calculate the *weight* per cent of gypsum (sp. gr. 2.32) and of anhydrite (sp. gr. 2.93) in the sample.

3. *Areal Analysis* (see page 443). — Examine a surfaced specimen of pearlitic steel, or copper containing Cu_2O, or a powdered mixture of chalcopyrite and chalcocite embedded in sealing wax. Illuminate by reflected light. Draw the outlines of the constituents on a sheet of coördinate paper, by means of a drawing camera, using the lowest magnification (largest field) which will permit the boundaries to be traced clearly.

Determine by counting squares the total area of each constituent in 10 different fields, and its per cent of the entire area drawn.

Calculate the weight percentage composition by the appropriate method:

a. From the specific gravities of the constituents (chalcocite, Cu_2S – 5.5; chalcopyrite, $CuFeS_2$ – 4.2).

b. From the known compositions of the constituents (pearlite = 0.85 per cent carbon; $Cu - Cu_2O$ eutectic = 0.35 per cent Cu_2O, or 0.39 per cent oxygen).

V. THE POLARIZING MICROSCOPE — CHAPTER IX

Test the polarizing microscope as directed on page 275, and in Experiments 1, 2, 3, and 4.

Study the general optical properties of doubly refractive materials, as directed in Experiments 5, 6, 7, 8, 10, 11, 13, 14, 15, 16, 18, 20. Write a brief discussion of your observations and conclusions.

VI. THE OPTICAL PROPERTIES OF CRYSTALS (see CHAPTER IX, pp. 308 *et seq*).

Study examples of the six crystal systems, recrystallizing as directed on page 343. Compare the properties observed (pages 315 to 322) with those given as typical of each system, and record them according to the outline on page 324.

VII. PHYSICAL CHEMISTRY OF CRYSTALLIZATION PHENOMENA — CHAPTER X

Perform the following experiments, recording your observations fully and making detailed drawings of the crystal forms obtained. Give a brief discussion of the physical chemistry underlying each experiment, and the conclusions which can be drawn from it.

Experiments: 1, a, b, or c; 3, a, b, or c; 6, a or b; 7; 8, b; 11, a, b, c; 12, a or b; 13, a, b, c, d, e; 14; 16, a. b, c; 17, a; 18; 19; 22, a, b, c, or d; 23, a, b, or c; 25, a.

VIII. HANDLING SMALL AMOUNTS OF MATERIAL — (VOL. II)

1. *Decantation.* Precipitate the following, by mixing small drops of the appropriate solutions, and decant the supernatant liquid, according to the method demonstrated by the instructor:

aluminum hydroxide
silver chloride
barium sulphate

2. *Filtration.* Filter a drop of a suspension of precipitated barium sulphate, according to the method demonstrated by the instructor.

3. *Testing Evolved Gas (or Distillation).* Using a tiny crucible, as demonstrated by the instructor, evolve ammonia from a minute particle of ammonium chloride, by means of sodium hydroxide solution, and collect it in a tiny drop of dilute hydrochloric acid. Identify the ammonium ion by means of chlorplatinic acid.

IX. ILLUMINATION AND CRITICAL MICROSCOPY

1. *Use of Condenser, Diaphragm, and Central Stop* (Chapter III). — Mount any well formed transparent material (crystals, starch grains, textile fibers) in water, and examine with the 16-mm. and with the 8-mm. objective.

a. Illuminate by transmitted light from the plane mirror (page 80), and note the character of the image as regards: *a*, contrast and intensity of shading; *b*, sharpness of outlines and fine details; *c*, ease of interpretation.

Render the illumination oblique, by swinging the mirror (page 82), and note the changes in the image.

b. Insert an Abbe condenser in the substage ring, and illuminate its aperture by means of an intense beam of parallel light. Place above it a cube of uranium glass, and observe the changes in the paths of the rays as the diaphragm opening is varied to give axial, convergent, oblique, or annular illumination (see below).

Illuminate the preparation by means of the condenser (page 84) having it focused on the plane of the object slide. Starting with the diaphragm fully opened, observe the changes in the character of the image as its aperture is gradually reduced. Observe the diaphragm opening as imaged at the back of the objective, and note for each objective the relative size of the illuminating cone when optimum contrast and detail are obtained.

Having the diaphragm fully opened, lower the condenser and observe the change in its effective illuminating cone (page 83) and in the character of the image.

Having the condenser in focus and the diaphragm fully opened, produce oblique illumination by screening one side of its aperture (page 85), and compare the character of the image with that obtained with axial or symmetrically convergent illumination. Next render the illumination as oblique as possible, so as to give a dark field (page 43).

Insert a central stop in the diaphragm opening, so as to furnish annular illumination with a dark field (page 86). Compare the character of the image with that obtained by bright field illumination. Explain what happens when the condenser is lowered.

2. *Critical Illumination and Resolution* (see page 44). Mount some diatomaceous earth in water. Adjust the microscope lamp so as to furnish diffuse light, and focus the condenser so as to image the light source in the plane of the preparation. Regulate the diaphragm for maximum visibility, as above, using a magnification of at least 200×. Search the preparation for diatom fragments which show markings so fine as to be *barely* resolvable. Then close the diaphragm as far as possible, and note the effect upon: *a*, contrast; and *b*, detail in the image. How does opening the diaphragm affect the image? How does lowering the condenser affect the image?

3. *Dark Field Illuminator* (see page 86).

a. Insert a dark-field illuminator in the substage ring, and illuminate its aperture with an intense beam of parallel light. Place above it a cube of uranium glass. Explain why no light leaves the illuminator. Then make optical contact between the glass and the illuminator, by means of a drop of homogeneous immersion oil. Observe the paths of rays, and adjust the mirror so that they form a symmetrical hollow cone. Note the height of the apex of this cone above the top surface of the illuminator.

b. Completely remove the oil from the illuminator, and center it, as directed on page 90. Make optical contact with the object slide of the above preparation of diatomaceous earth. Adjust the mirror and focus the illuminator (page 91) so as to obtain a small circular illuminated field. Compare the character of the image with that produced with bright field illumination, as regards contrast, detail, ease of interpretation, and prominence of diffraction patterns. Note the Brownian movement of the tiniest particles.

4. *Oil Immersion Objective* (see page 24). — Prepare a drop of a uniform aqueous suspension of bacteria (which can be obtained by scraping the teeth with a sterile platinum loop). Spread a thin film of the suspension upon a *clean, greaseless* slide, and allow to dry at room temperature. The liquid should not collect into drops, but should evaporate to leave the bacteria evenly distributed. When dry, fix the preparation by passing, film side up, three times through the flame of a Bunsen burner. Cover the film with an aqueous solution of Gentian Violet, and allow the stain to act for two or three minutes. Wash off the excess stain, dry the slide except for the film of bacteria, and place a clean cover-glass on it, having a *thin* layer of water as the mounting medium.

Examine the preparation with a 16-mm. and with an 8-mm. objective, using the condenser. Replace the 32-mm. objective by an oil immersion objective, place a drop of immersion oil on its front lens, and focus very cautiously, so as to avoid pressing on the cover-glass. Note the high resolving power and very slight depth of focus of the objective.

5. *Aberrations* (see pages 17, 19). — Spread a small quantity of a very fine paint pigment evenly upon an object slide, by the method given on page 170. When the dispersing medium has evaporated, examine the preparation with the 8-mm. objective, using the condenser. Note that the particles are not resolved, although visible as single dark points. Observe the lack of

flatness of field of the objective (page 18), and the colored border of the field due to chromatic aberration in the eyepiece (page 33).

Examine the uncovered preparation with a 4-mm. "dry" objective corrected for use with a cover-glass. Adjust the condenser diaphragm to give the best definition, and note that at no position of focus are the outlines of the particles sharp and free from blurring. Cover them with a cover-glass, and observe the improvement in the sharpness of their outlines (page 25) and in resolution. Notice the marked curvature of field, and the residual color due to incompletely corrected chromatic aberration (page 19).

Place a very thin film of water between the object slide and the cover-glass, and examine the water-mounted preparation with an oil immersion objective, paying particular attention to the quality of the image.

6. *Vertical Illumination* (see page 116). — Mount a metal specimen in "Plasticene" on an object slide, so that its prepared surface is perpendicular to the axis of the microscope. Attach a mirror-type vertical illuminator (page 121) in place of one of the objectives and screw into it a 16-mm. objective. Slide the small mirror to one side of the back aperture of the objective and focus on the specimen, so as first to examine its appearance with inclined illumination (page 114). Note which portions appear dark and which bright.

Align the light source with the side tube of the illuminator, and place the mirror so as to send light downward through the objective. Adjust the position of the mirror so as to give an evenly illuminated field, and compare the appearance of the specimen with that obtained under inclined illumination.

X. REFRACTIVE INDEX — CHAPTER XI

1. Perform the following experiments, making drawings of the appearances observed and recording in detail the method by which the illumination was obtained, in each case:

Experiments: 1, a, b; 2; 3, a, b, c, d, e.

2. Determine the two refractive indices ϵ and ω, of a uniaxial substance (see pages 292, 375) and indicate their position by a drawing.

3. *Refractive Index of Liquids* (see page 380). — Prepare a curve according to Method 2 (page 383), using the following liquids: $n = 1.333$ (water); $n = 1.445$ (kerosene); $n = 1.600$ (oil of cassia).

Determine the refractive index of an unknown, by the above method.

XI. COMMON TEXTILE AND CORDAGE FIBERS (see page 447)

Place a little of the material in a drop of water on a slide, and allow to soak for a few minutes. (It should be borne in mind that moisture causes most fibers to swell and changes their shapes somewhat.) Separate the individual fibers carefully by means of dissecting needles, so as to isolate the ultimate component fibers or cells. Cover the fibers with a cover-glass and study their morphological characteristics, first at low magnification and then with a higher-power objective. Employ oblique as well as axial illumination, and vary the diaphragm opening, in order to obtain the best conditions for revealing the various details of the fibers. Examine between crossed nicols, and note how variations in thickness, twists, and dislocations or "nodes" are revealed. Ascertain the "order" of the polarization colors of *single* fibers (page 280).

Make drawings, all approximately to the same scale, of at least *three* typical fibers of each material. The diameter of the individual fibers as drawn should not be less than 5 mm., and as much of the length as possible should be shown. The cross-section should also be indicated. Show the various characteristic structural features, and supplement the drawings by a brief description.

The most important and distinctive characteristics, which should be sought in each case, are given as a guide to study:

SEED HAIRS

Cotton (*Gossypium*). Fibers unicellular, 3 cm. or more in length. Collapsed tubes, thick-walled except in unripe hairs. Edges often thickened. Central canal closed, or air-filled in places. Very pure cellulose.

Mercerized Cotton (Cotton treated with caustic soda). More nearly cylindrical and less twisted than ordinary cotton. Smoother fibers than unmercerized cotton.

Kapok (*Eidendron* or *Bombax*). Thin-walled, cylindrical fibers, 1–3 cm. long, which taper to a point at the tip. Bulbous, reticulated bases. Very brittle, and collapse on bending. Many air bubbles entrapped in the central canal, when mounted in water.

BAST FIBERS

Flax (*Linum*). Fibers unicellular, 2–3 cm. long. Occur in compact bundles, several fibers thick and 30–100 cm. in length. Individual fibers polygonal to circular in cross-section, with gradually tapering ends. Central canal broad in some fibers, in others very narrow or almost invisible. Cells are made up of very minute fibrillae, which are sometimes noticeable in frayed, split, bruised, or broken fibers, or as faint longitudinal striations. These fibrillae are sharply deformed or dislocated if the fiber is bent abruptly, resulting in characteristic "nodes" or cross-markings, which are more frequent and prominent in "worked" fibers such as worn linen. The nodes, on account of their discontinuity with the rest of the fiber, are best revealed between crossed nicols. Very pure cellulose, especially after bleaching.

Hemp (*Cannabis*). Structurally very similar to flax, and distinguishable from it with difficulty. Fibers of hemp are likely to be coarser and more flattened with broader lumens, forked ends, and more prominent longitudinal striations. Treatment with reagents which stain or swell the fibers is the best means of differentiating them from flax. The fibers are almost pure cellulose, but a slight amount of lignin is present.

Ramie or "*China Grass*" (*Boehmeria*). Fibers unicellular, 6–25 cm. in length, occurring in bundles and singly. Flattened in cross-section, and of widely varying sizes, averaging much coarser than flax or hemp. Central canal usually broad and distinct. Nodes and dislocations well marked. Longitudinal fissures prominent. Fibers stiff and brittle with little tendency to split or fray without breaking. Cellulose almost perfectly pure and free from lignin.

Jute (*Corchorus*). Fibers unicellular, a few millimeters in length, occurring in compact bundles often 1–2 meters long and many fibers in cross-section. Cells tapering, with pointed ends, and a well defined central canal which varies from more than half the diameter of the fiber to a mere line, and is often completely interrupted. Polygonal cross-section and faint longitudinal striations but no nodes or dislocations. Rather brittle, with little evidence of fibrillae. Cellulose markedly lignified.

LEAF FIBERS

Manila or "*Abaca*" (*Musa*). Fibers unicellular, gradually tapering and 5–10 mm. in length, occurring in compact bundles which may be 1–4 meters long. Cells oval or circular in cross-section, with rather smooth surface and well defined uniform central canal, often half the diameter of the fiber. Fibers tough and flexible, without nodes. Degree of lignification varies with the individual fibers.

Sisal (*Agave*). Fibers unicellular, 2–5 mm. in length and distinctly tapering. Occur in compact bundles about 1 meter long, and many fibers thick. Cells polygonal or rounded in cross-section; central canal well defined and often broader than half the diameter of the fiber. Fibers rather brittle, without nodes. Shorter and thicker cells, with oblique surface fissures, are often present. Cellulose contains considerable lignin.

SYNTHETIC FIBERS

Viscose Silk (Cellulose; prepared by extrusion of cellulose xanthate into a coagulating solution). Continuous, separate, rather coarse fibers, with deep longitudinal striations; serrated and usually oval flattened or crescent-shaped in cross-section, striations. Moderately strong birefringence. Marked swelling on immersion in water.

Acetate Silk (Cellulose acetate; extruded into a coagulating medium). Continuous separate rather coarse fibers; usually oval or crescent-shaped in cross-section, with almost smooth surfaces. Very weak birefringence.

Collodion Silk (Cellulose; from nitrocellulose extruded and de-nitrated). Continuous, separate, rather coarse fibers, often with thickened edges; oval, crescent-shaped, or irregular in cross-section. Smooth surfaces; structurally similar to acetate silk. Rather strong birefringence.

Cuprammonium Silk (Cellulose; prepared from solution in ammoniacal cupric oxide, by extrusion into a coagulating liquid). Continuous, separate, nonstriated filaments, often much finer than the other synthetic fibers. Round or oval in cross-section. Moderately strong birefringence.

NATURAL SILKS

Silk (" Mulberry Silk "). From the domesticated worm (*Bombyx mori*) which is fed on mulberry leaves. The silk is extruded by the worm from two glands, and hardens to give a filament consisting of two strands or " brins." A coating of white or yellow " gum " (sericin) is formed upon the fibers as they are spun, and serves to cement them together into a double " bave." The cocoons are soaked in warm water, and several are unwound simultaneously; their filaments are reeled together to give " raw " silk, which consists of 18–40 fibers cemented by sericin to form a stiff thread. The sericin may be removed by washing to give " degummed " silk. Floss silk consists of short waste fibers not reeled together.

The individual silk fibers are continuous, fine, smooth filaments, of nearly uniform diameter except where flattened by other fibers in the spinning of the cocoon. The fibers are very strong and elastic, and highly flexible. Their cross-section is irregularly oval; longitudinal striations are faint or lacking. The original pairs of fibers are rare in degummed silk or finished fabrics. Birefringence is fairly strong.

" *Tussah* " or " *Wild* " *Silk*. From other varieties of worms than *Bombyx*. Slightly brownish in color (as in natural " pongee " fabrics).

Fibers are continuous ribbon-like filaments, much broader and less uniform than those of " mulberry " silk, with many fine longitudinal striations. The cross-impressions from contact with other fibers in the spinning of the cocoon are clearly evident as double imprints, usually crossing the fiber obliquely. Cross-sections are flattened or triangular. Birefringence is fairly strong.

ANIMAL HAIRS

Wool. From many varieties of sheep. Individual elastic fibers, 3–15 cm. long, more or less finely " crimped " or wavy, round in cross-section, and of uniform diameter. The fibers are covered with overlapping scales, beneath which is a cortex of elongated and compacted cells. Coarser hairs (" Kemps ") may also be present; these possess a more or less continuous medullary canal which is filled with granular or pithy material, and which may be of such large diameter as to render the fiber weak, brittle, opaque, and difficult to dye.

The varieties of wool differ in the length, diameter and waviness of the fiber, in their freedom from Kemps, and in the shape, size, and arrangement of the scales.

Mohair. From the Angora goat. Individual elastic fibers, 15–50 cm. in length, practically straight, round in cross-section and uniform in diameter. The diameter of different hairs may show considerable variation, and "Kemps" are common. The scales are thinner and more closely adherent than in wool; the longitudinal cells of the cortex are clearly visible beneath them.

XII. COMMON PAPER FIBERS (see page 447)

Place a small sample of the paper in a drop of water on an object slide, and allow to soak until thoroughly permeated and softened. Then scrape *lightly* with the blade of a "spear point" dissecting needle, so as to remove individual fibers (*not* tiny bits of paper) and yield a bulky mass of loose pulp in the water. Spread out the pulp, cover with a cover-glass, and study the morphological characteristics of the fibers, first at low magnifications and then as regards their finer details of structure. Vary the conditions of illumination so as to obtain optimum visibility. Examine between crossed nicols, and note the "order" of the polarization colors and the appearance of the structural features.

Make drawings, approximately to scale, which show all the different kinds of typical fibers and cells in each specimen, and which indicate their length and their degree of separation from each other. Supplement the drawings by a description of the distinctive features observed.

Dry the pulp, by pressing the fibers against the slide with a piece of filter paper. Add a drop or two of Herzberg's stain,[1] and disperse the fibers evenly in it by raising and lowering the cover-glass.

Prepare a pulped mixture of several different kinds of fibers, and stain as above, noting the color of each ingredient.

The following colors are given by Herzberg's stain:

Yellow or yellow-green: lignified cellulose, such as ground wood, straw, manila, jute.

Blue, gray or blue violet: purified cellulose, such as chemical wood pulp, esparto, bleached straw, jute or manila.

Wine red: pure cellulose, such as cotton or linen rags.

In the examination of paper fibers particular attention must be paid to: (*a*) their inherent morphological features, and (*b*) their physical and chemical condition as governed by the manufacturing process to which they have been subjected. The following outline will serve as a guide to the characteristics which should be sought in microscopical study:

Chemically Prepared Wood Pulps. Fibers almost perfectly separated, by cooking chipped wood in solutions of calcium bisulphite ("sulphite" process), sodium carbonate ("soda" process), or alkaline sodium sulphide ("sulphide," "sulphate," or "kraft" process). Individual cells intact, unless broken by preparing for examination, or frayed by "beating" in the manufacture of pulp. Fibers long, flexible and easily interlaced to form paper. The purity of the cellulose varies with the process employed and the amount of bleaching; the lignin is largely removed.

Mechanically Prepared Wood Pulps ("Ground Wood"). Blocks of wood are torn into fine shreds by grindstones under water, giving fibers which are torn, broken, and often imperfectly separated, so that groups of cells are common. The fibers are relatively short and stiff, and not readily entangled

[1] Herzberg's stain is prepared by dissolving 50 g. of zinc chloride (fused sticks) in 25 cc. of water, adjusting the specific gravity of the solution to 1.8 at 28° C. To this solution is added a solution of 5.25 g. of potassium iodide and 0.25 g. of iodine in 12.5 cc. of water. After standing over night the clear portion is decanted, and a crystal of iodine is added to it. The stain should be kept in a dark bottle, and should be tested with a mixture of known fibers, especially if not freshly made.

together to form strong paper. The cellulose is subjected to almost no purification, and a large amount of lignin is present.

Coniferous Wood Pulp. The condition of the fibers depends on whether they are prepared chemically (usually by the sulphite or the sulphide process) or mechanically. Several types of cells are present; the most characteristic are long thin-walled single or double tubular cells or tracheids, polygonal in cross-section and marked by single or double rows of irregularly spaced *bordered pits;* and tapering fibers, often with fairly thick walls, and with faint oblique fissures or surface striations; small, flat, rectangular cells (from " medullary rays ") often with very tiny pits are also typical. The details of the various cells serve to differentiate between the spruce, fir, pine, hemlock and other woods from coniferous trees.

Non-Coniferous Wood Pulp. Separation and intactness of cells dependent on whether chemical (soda process) or mechanical preparation is used. Long, tapering fibers, thin-walled and with almost no surface markings, make up the bulk of the pulp; very large " *vessels*," several times the diameter of the fibers, and more or less completely covered by many rows of small pits or perforations, are particularly characteristic. The detail of the pitting and the shape of the vessels serves to differentiate between poplar, bass, beech, birch, maple, or other woods of " broad-leaved " or deciduous trees.

Rag. Cotton or linen fibers, usually frayed and torn from beating in the manufacture of pulp. The twisted cells of cotton and the nodes of flax may usually be recognized, but many fibers retain practically no characteristic structural features, and must be identified by staining. Rag pulp consists of almost perfectly pure cellulose.

Esparto. The bulk of the pulp (prepared by the soda process) consists of very slender cells, with gradually tapering pointed ends. Short flat rectangular cells with serrated outlines are fairly common; small, stubby hairs, often more or less comma-shaped, are characteristic, though easily overlooked. The cellulose is almost completely purified, but the degree of lignification of the individual fibers varies.

Straw. Sometimes prepared by the soda process, giving well separated and highly purified fibers; more commonly pulped by a brief cooking with calcium hydroxide solution, which yields imperfectly separated groups of cells, deep yellow in color and highly lignified. Long tapering bast fibers, with a small central canal, are most frequent; small, flat, rectangular cells with deeply serrated interlocking edges, and large thin-walled oval cells are particularly characteristic. Spiral vessels and other types of cells also occur.

Jute, Hemp, and *Manila* are often used in paper. Their structural features are as described above, except that a certain amount of fraying may be noticeable, and the lignification of the fibers is likely to be less in pulp than in the raw and unbleached state.

XIII. LABORATORY DEMONSTRATION OF SPECIAL APPARATUS AND METHODS

Ultramicroscopy; photomicrographic methods; metallographic microscopes; preparation of materials for microscopic study.

KEY TO BLOCK CONTAINING MATERIALS FOR EXPERIMENTAL WORK IN COURSE IN INTRODUCTORY CHEMICAL MICROSCOPY

Cornell University — Department of Chemistry

C530

	1	2	3	4	5	6	7	8	9	10	11	12	
A	Sodium Chloride	Ammonium Chloride	Potassium Iodide	Barium Nitrate	Lead Nitrate	Ammonium Alum	Ammonium Dihydrogen Phosphate	Mercuric Cyanide	Potassium Copper Chloride	Urea	Sodium Nitrate	Strontium Antimonyl Tartrate	A
B	Iodoform	Thymol	Silver Nitrate	Ammonium Sulphate	Potassium Nitrate	Ammonium Perchlorate	Potassium Permanganate	Borax	Barium Chloride	Potassium Chlorate	Copper Acetate	Ferrous Sulphate	B
C	Sodium Thiosulphate	Copper Sulphate	Potassium Bichromate	Gelatine	Stearic Acid	Sulfonal	Ortho-nitrophenol	Naphthalene	Phthalic Anhydride	Benzoic Acid	Indigo	Mercuric Iodide	C
D	Carbon Tetrabromide	Mono-Chloracetic Acid	Zinc	Rice Starch 5% Potato	Rice Starch 10% Potato	Rice Starch 20% Potato	Diatomaceous Earth	Non-Coniferous Wood Mechanical	Coniferous Wood Sulphite	Rag	Manila	Esparto	D
E	Cotton	Flax	Hemp	Jute	Ramie	"Viscose-Silk"	"Acetate Silk"	"Cuprammonium Silk"	"Mulberry Silk"	Wild Silk	Wool	Mohair	E
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